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JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

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ERRATA AND AUTHORS' EMENDATIONS

- Page 43, Table XVI, last figure in column headed "Number of varieties found susceptible" should be "1" instead of "21."
- Page 72, line 17, "eugenol" is misspelled.
- Page 74, Table I, last column, ".338" should be "33.8."
- Page 76, Table III, sixth line from bottom, "Eugenol" is misspelled.
- Pages 393-394, curves of Figures 2 and 3 should be interchanged.
- Pages 580, 581, 582, Table I, footnote "c" applies to rows 28 and 38 only.
- In footnote "d" row No. "44" should be "43." Footnote "a," page 582, should be the same as footnote "d" on preceding page.
- Page 603, line 19, the word "data" should follow "climatological."
- Pages 622 and 626. The graphs of Figures 1 and 3 should be interchanged, but the legends and text references are correct as given.
- Page 629, line 11, "varieties of lettuce" should be "varieties of plants."
- Page 686, tenth line from bottom, "nineteenth" should be "seventeenth."
- Page 723. Insert in line 10 between "Sacc.," and "and" within brackets "[*Ophiobolus graminis* Sacc.]"
- Page 724, seventh line from bottom, "Prenophora" should be "Pyrenophora."
- Page 773, line 11, a comma and "or" should be inserted between "prematurely" and "dead."
- Page 777, seventh line from bottom, "percentages" should be "percentage."
- Page 789, third line from bottom, "effected" should be "affected."
- Page 841, seventeenth line from bottom, "Rapid" should be "Rapp."
- Page 859, lines 2 and 3, insert "spongy" before "mesophyll."
- Page 862, line 2 of third paragraph from bottom, insert "spongy" before "mesophyll."
- Page 917, line 9, substitute "the host" for "it."
- Page 963, line 16, "are" should be "as."
- Page 1001, first paragraph, last line, "days" should be "data."
- Page 1005, Table III, under "Constituent," "Purine" should be "Purines."
- Page 1070, second line from bottom, "II" should be "III."
- Page 1071, line 28, "(I and II)" should be "(I and III)."
- Page 1071, line 29, "(III)" should be "(II)."
- Page 1078, eleventh line from bottom, "4.424" should be "4.425."
- Page, 1191, 1193, 1195, 1197, and 1199, the running head at top of page should read: "Influence of Nitrate Nitrogen on Wheat Protein and Yield."

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VOL. XXXI WASHINGTON, D. C., JULY 1, 1925

No. 1

FURTHER STUDIES ON THE OVERWINTERING AND
DISSEMINATION OF CUCURBIT MOSAIC¹

By S. P. DOOLITTLE, *Pathologist*, and M. N. WALKER, *Junior Pathologist*,² Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Earlier studies of the mosaic disease of cucurbits have shown that a knowledge of the agencies by which the disease lives over winter is essential to the development of efficient control measures. The results reported in previous papers (5, 8)³ indicated that the soil was not a factor in overwintering the disease and that the seed of the cultivated cucumber, *Cucumis sativus* L., was rarely, if ever, a source of infection. There was also no evidence that the striped cucumber beetle, *Diabrotica vittata* Fabr., was an agency in the overwintering of mosaic. There was evidence, however, that the disease might overwinter on wild host plants, since it was found that seeds from mosaic plants of the wild cucumber, *Micrampelis lobata* (Michx.) Greene, would produce a certain percentage of mosaic diseased seedlings (?). Continued investigations during the past three years have shown that cucumber mosaic is transmissible to a number of plants of other families and have established the importance of certain wild host plants in the overwintering of the disease.

It is the purpose of this paper to present the evidence obtained from numerous experiments which have led to these conclusions.

SOIL IN RELATION TO OVERWINTERING

Experiments in Wisconsin and Michigan, prior to 1919 (8), indicated that cucurbit mosaic was not carried in the soil, and the results of further trials during 1919 and 1920 have strengthened this conclusion. All of these later experiments were conducted at Madison, Wis., on the same land as that used in the trials of 1917. This plat had grown cucumbers for three successive years, and the mosaic disease had been severe there each season.

EXPERIMENTS OF 1919

The plat was planted to cucumbers on June 19, using commercial seed of the Chicago Pickling variety. Ten cheesecloth cages of the

¹ Received for publication August 20, 1924; issued September, 1925.

² The writers are indebted to W. W. Gilbert, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, for valuable suggestions and advice during the progress of this work and for assistance in obtaining certain field data and material used in these studies. They are also indebted to L. R. Jones, of the Department of Plant Pathology of the University of Wisconsin, for advice and criticism during the course of this work.

The Department of Plant Pathology, University of Wisconsin, has cooperated in furnishing land and laboratory and greenhouse facilities necessary for these investigations, and certain interested pickle companies and growers have cooperated by furnishing funds and facilities for carrying on the work on eradication and control in the field.

³ Reference is made by number (*italic*) to "Literature cited," p. 57.

type used in the earlier experiments (8) were put down at the time the seed was planted and 10 more were placed over other plants on June 29. Care was taken to see that these plants were free from insects before caging and the cages were lifted only once thereafter, on July 1, in order to thin the plants. The 20 cages, covering a total of 120 plants, were removed on August 13, when the vines had become so large so as to begin to tear the cloth. The caged plants were examined and all were found to be free from mosaic. On the same date approximately 95 per cent of the 2,000 uncaged plants in the plat were mosaic-diseased.

EXPERIMENTS OF 1920

A similar experiment was conducted in 1920, using the same plat but planting a different variety of cucumbers in each row. The cages were not put down until later in the season, however, owing to the necessity of replanting most of the field on June 29. Eighteen cages were used, seven of which were put down on July 1 and the remaining eleven on July 9, when the plants were just above ground. Many of the caged plants showed injury from the striped cucumber beetle, but great care was taken to exclude these insects from the cages.

On August 2 some of the cages were lifted and the plants thinned where necessary, reducing their number from 184 to 108. At this time approximately 9 per cent of the uncaged plants in the plat were mosaic-diseased. No mosaic was found on any of the caged plants, although the cages put down on July 9 contained a number of plants affected with bacterial wilt. On August 16 the cages were finally lifted and the plants carefully examined for signs of mosaic infection. They proved healthy in all cases, while only 14 mosaic-free plants were found in a total of 2,100 plants left uncaged in the plat.

These results, combined with those earlier reported (8), have shown that plants protected from insects may be grown year after year on the same soil and remain absolutely free from mosaic infection in all cases, although nearly all of the uncaged plants may become infected each season. In view of these results, it seems definitely established that the soil is not an agency in overwintering cucurbit mosaic.

TRIALS OF SEED FROM MOSAIC PLANTS OF THE CULTIVATED CUCURBITS

CUCUMBER

Trials made with seed from mosaic cucumber plants prior to 1919 (6, 8) indicated that mosaic infection, through the seed of the cultivated cucumber, occurred very rarely, if at all. Only one case of apparent seedling infection had occurred during trials of 10,500 plants grown from seed from mosaic cucumber fruits, but the single case was so well substantiated that it assumed a possible importance, inasmuch as the disease is transmitted by insects so rapidly that a single mosaic plant may serve as a source of infection for all the fields in the vicinity. In order to obtain more conclusive results, therefore, the tests were continued both in the field and in the greenhouse.

FIELD TRIALS OF 1919

The seed used in these trials was collected by M. W. Gardner at Grass Lake, Mich., and Sparta, Wis., in the summer of 1918, from fruits which showed symptoms of the mosaic disease. The seed from each fruit was removed and planted as a separate lot. The trial plat was located at Ellison Bay, Wis., in the northern part of Door County, where mosaic was not known to occur and where cucumbers were not planted extensively. Approximately 10,000 seeds from 77 fruits were planted on June 20, but owing to the low percentage of germination, only 3,400 plants were obtained. These plants were kept under observation during the summer and inspections of the individual plants were made on July 11 and 26. Each plant was carefully examined and inoculations were made from 16 plants which showed abnormalities of growth or leaf color. None of these inoculations produced mosaic and no evidence of the disease appeared on any of the plants during the season.

GREENHOUSE TRIALS

Studies of the relation of temperature to the development of cucurbit mosaic have shown that the disease develops most rapidly at temperatures of about 30° C., and it was decided, therefore, to conduct trials of seed from mosaic plants at the temperature which seemed most favorable to the development of the disease.

TRIALS OF 1920

Seed collected from mosaic cucumber plants at Madison in 1919 was planted during the winter in two greenhouses held at temperatures of approximately 24° and 30° C., respectively. The temperature was kept reasonably constant within limits of 2° in the house at 30° and with a variation of 5° in the other house. Plants from the seed of each fruit were grown in each of these houses, the two temperatures representing the optimum for the disease at 30° and an approximate optimum for the plant at 24°. The seeds were planted in duplicate flats of steam-sterilized soil, each flat containing 25 seeds from each of four fruits. A total of about 3,500 seedlings were obtained from the seeds of 77 fruits during the winter. The plants were kept until three leaves developed and were allowed sufficient room in the flat for normal growth. No other cucurbits were grown in the greenhouse used for these experiments and the houses were fumigated regularly in order to guard against infection by insects. No mosaic developed during these trials and the plants made normal growth in all cases.

TRIALS OF 1921

During the winter of 1920-21, further trials were carried on in the greenhouse, using seed from the same source and growing seedlings from each fruit in duplicate series at air temperatures of approximately 30° and 24° C. Two series were conducted during the winter and early spring, a total of 2,200 plants being under observation during this time. The plants made normal growth and failed to show any symptoms of mosaic during the trials, although they were kept until the third leaf was well developed.

MUSKMELOON

GREENHOUSE TRIALS OF 1920-21

In addition to the trials with seed from mosaic cucumber plants, experiments were also conducted with seed from mosaic muskmelon plants, *Cucumis melo* L. As in the case of the cucumber, there had been field observations which suggested the possibility of seed transmission of the disease (8), but no actual experimental data had been obtained. Seeds from mosaic muskmelon plants were collected at Madison, Wis., during the summer of 1919, and an additional supply collected at Vincennes, Ind., was received from M. W. Gardner, of the Indiana Agricultural Experiment Station.

The trials were made in the greenhouse at Madison, following the same procedure as that described above in the case of the cucumber. Several series were conducted during the winters of 1920 and 1921, using seed from the same lot. The seeds were grown in duplicate series at air temperatures of 24° and 30° C. and the plants were kept under observation for approximately 30 days. Under these conditions 2,700 plants were grown until the third leaf had formed, but no symptoms of mosaic were noted on any of the plants grown at either temperature.

SQUASH AND PUMPKIN

GREENHOUSE TRIALS

Although the trials with both the cucumber and muskmelon have yielded evidence almost wholly negative in regard to seed transmission of mosaic, seeds from mosaic plants of the wild cucumber, *Micrampelis lobata*, have been shown to be carriers of the disease (7). In view of this peculiar circumstance, it was thought that the seeds of the larger-seeded cucurbits, such as *Micrampelis*, might be more likely to carry the virus than those of the smaller-seeded type, such as the cucumber and muskmelon. Experiments were therefore carried on in the greenhouses at Madison during the winter of 1921, in which seed from mosaic fruits of certain varieties of squash and pumpkin were grown in flats of sterilized soil at temperatures of 24° and 30° C., as in the earlier trials with seed from the cucumber and muskmelon.

The seed used was collected during the summer of 1920 from mosaic fruits of the Large Cheese pumpkin, *Cucurbita moschata* Duchesne, the Summer Crookneck and Golden Custard squashes, *Cucurbita pepo* L., and the Hubbard squash, *Cucurbita maxima* Duchesne. The following numbers of plants were grown from seed from the different varieties:

Summer Crookneck squash.....	475
Golden Custard squash.....	270
Hubbard squash.....	190
Large Cheese pumpkin.....	180

All of these plants were held until the third leaf had developed, and the growth, although somewhat elongated, appeared to be of normal character. No indications of mosaic were found on any of the plants of these varieties.

The accumulated evidence of all trials with seed from mosaic plants of the cultivated cucurbits has been so overwhelmingly negative that it seems evident that the seed of the cultivated varieties is

not an agency which can be considered important as a means of overwintering the mosaic disease. Only one case of apparent seedling infection has occurred in trials with approximately 22,000 cucumber plants grown from seed from mosaic plants, and none in the less extensive trials with muskmelon, squash, and pumpkin. Aside from this single instance there have been perhaps five instances of possible seedling infection from commercial cucumber seed and perhaps one in the case of the muskmelon. In such cases, however, there has usually been considerable doubt as to the actual source of infection. In any case, the rarity of such infection has been demonstrated and, in view of the prevalence of other sources of primary infection, the seed of the cultivated cucurbits seems a negligible factor. It should be noted, however, as will be shown later, that the seed of the wild cucumber, *Micrampelis lobata*, is an important agency in overwintering the disease.

THE STRIPED CUCUMBER BEETLE AS A FACTOR IN OVERWINTERING MOSAIC

Studies of the striped cucumber beetle, *Diabrotica vittata*, in relation to the overwintering of mosaic, which had been in progress prior to 1919 (8), were continued during 1919 and 1920. The major portion of this work was in cooperation with J. E. Dudley, jr., of the Bureau of Entomology, United States Department of Agriculture. The collection and studies of the hibernation and life history of the insect were under his direction, and the studies of the beetles as possible carriers of the mosaic virus were conducted by the writers.

TRIALS WITH BEETLES FROM HIBERNATION CAGES

During the fall of 1918, several thousand beetles were collected by Dudley and placed in hibernation cages at various points. The beetles were fed on mosaic-diseased leaves and fruits of cucurbits as long as such material was available. In the latter part of April, 1919, 392 beetles which survived the winter were obtained from two of these cages and allowed to feed on healthy cucumber plants placed under cheesecloth cages in the greenhouse. Fifty small cages were placed over individual plants and a single beetle was placed in each cage. The remaining beetles were divided into lots of 25 to 80 and placed in larger cages containing several cucumber plants. In a few of the cages, young plants of *Micrampelis lobata* were included with the cucumbers. The beetles thus fed on 120 cucumber plants and 21 plants of *Micrampelis*. The plants were removed from the cages after an interval of four to eight days and held for at least two weeks for observation, but no signs of mosaic infection developed in either species.

EXPERIMENTS WITH BEETLES COLLECTED IN THE OPEN

At the time the beetles emerged from hibernation in the spring of 1920, a considerable number were collected in the open fields and fed on healthy cucumber plants under cages in the greenhouse. Between April 21 and May 10, 292 beetles were thus obtained and allowed to feed in cages containing 85 cucumber and 22 *Micrampelis* plants. No sign of mosaic appeared on any of these plants, although they were under observation for four weeks.

In addition to this 80 cucumber plants were set out on May 20 in the vicinity of cucumber fields where mosaic had occurred during the previous season. Several groups of *Micrampelis* plants, 110 in all, were also planted about the edge of a field where the disease seemed to occur earliest each season. All of these plants were attacked by the beetles to some extent, but none of them became infected with mosaic until late in the season.

Further trials were made during the spring of 1921 with 452 beetles collected in the neighborhood of cucumber fields between May 25 and June 4. The insects were fed on healthy cucumber plants in the same manner as in previous trials, a total of 176 plants being used in the experiments. All these plants remained healthy.

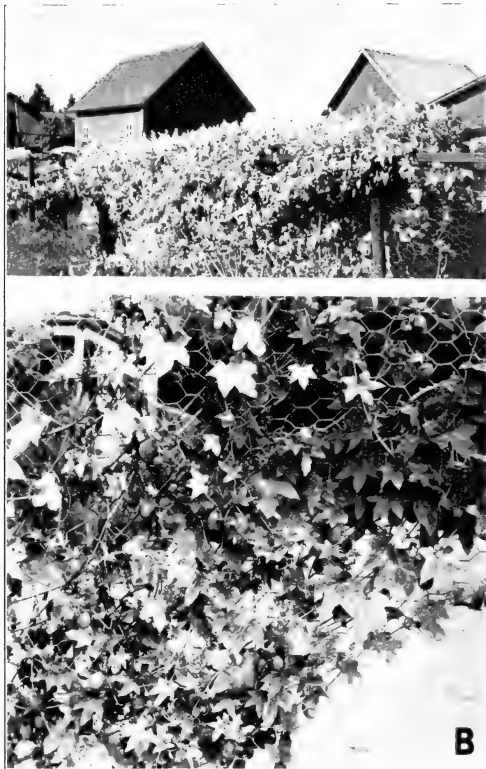
The results of these and earlier experiments (8) indicate that the beetle does not carry the disease through the winter, and the evidence is strengthened by experimental data which indicate that after feeding on mosaic plants they carry the virus for only a short period. The trials with beetles from hibernation cages were, however, the only ones of definite value in this regard, since it has been found that the wild cucumber, on which the insects feed after emerging from hibernation, is often affected with cucurbit mosaic. Infection obtained from beetles taken in the open, therefore, is not conclusive evidence that they carry the disease overwinter.

OVERWINTERING OF THE MOSAIC DISEASE ON WILD HOST PLANTS

Since the seed of the cultivated cucurbits seems practically eliminated as a carrier of cucurbit mosaic, and since neither the soil nor insects appear to be concerned in overwintering the disease, attention has been focused on the possible existence of wild host plants which carry the disease from year to year and serve as sources of primary infection to the cultivated cucurbits. As a result of these studies, it has been found that the wild cucumber, *Micrampelis lobata*, the milkweed, *Asclepias syriaca* L. (9), the poke weed, *Phytolacca decandra* L. (10), and probably certain *Physalis* spp., are important factors in overwintering the disease.

RELATION OF THE WILD CUCUMBER TO THE OVERWINTERING OF CUCURBIT MOSAIC

Efforts to connect the wild cucumber, *Micrampelis lobata*, with the overwintering of mosaic arose from the fact that this plant is common throughout the cucumber-growing sections of the Middle West and is affected with a mosaic disease identical in appearance with that occurring on the cultivated cucurbits. Furthermore, the wild cucumber is frequently used as an ornamental vine on fences and porches and is often found in the vicinity of cucumber fields (pl. 1, A). Observations made during 1917 and 1918 (8) showed that wild cucumbers growing at certain points became mosaic-diseased year after year and that the disease appeared on these plants considerably earlier than in adjacent cucumber fields. As the wild cucumber is an annual, it was thought that the disease in these cases must be carried in the seed, although there was little evidence of seed transmission in the case of the cultivated cucumber. An extensive trial of seed from mosaic *Micrampelis* plants was made in 1918, but, owing to unfavorable greenhouse conditions, the results were negative (8). A further trial during 1919 (7), however, gave apparent evidence of seed transmission which has been confirmed by the results of the additional tests during the last three years.



HEALTHY AND MOSAIC WILD CUCUMBER PLANTS

- A. Healthy wild cucumber plants, *Micrampeles lobata*, showing typical habit of growth during flowering period. Ellison Bay, Wis. July, 1919
B. Mosaic wild cucumber plants showing mottling of foliage

TRIALS WITH SEED FROM MOSAIC PLANTS OF WILD CUCUMBER

The methods followed in the experiments with seed from mosaic plants of *Micrampelis lobata* were approximately the same in all trials. The seed was collected from plants which showed definite mosaic symptoms and, in most instances, was obtained from points where mosaic-diseased plants had appeared during previous seasons. Seed was also collected from healthy plants to serve as controls.

The seeds were dried at room temperature and then stratified in moist sand and held for a time at low temperatures. In the earlier work with the wild cucumber, considerable difficulty was encountered in obtaining germination of the seed, until, as a result of numerous experiments carried out by G. H. Harrington, formerly of the seed laboratory of the United States Department of Agriculture, it was definitely shown that a period of chilling is essential to its germination. In the trials of 1919 the seeds were stratified in a box of moist sand and allowed to remain out of doors during the winter, being brought in and planted on April 9. In the later trials the seeds were, in some cases, treated in the same way, but most of them were packed in moist sand and placed in the ice compartment of a refrigerator. Under these conditions, a period of three to five weeks was necessary for germination. A few seeds were tested for germination at room temperature at intervals of 10 days, but it was found that the seed remaining on ice would germinate as rapidly as the test samples removed to higher temperatures. When germination began, all the seeds were removed from the ice compartment and placed in moist chambers to germinate. Seeds which did not germinate within five days were again packed in sand and subjected to further chilling. Practically all the seeds which eventually germinated, however, did so within three to five days after germination was first noted, regardless of the temperature at which they were subsequently held. Little germination occurred in any of the remaining seed, although they were often kept at low temperature for six weeks thereafter.

In all trials of the seeds from mosaic *Micrampelis* plants, the average percentage of germination was much lower than that of the healthy control seeds which had been collected at the same time and kept under the same conditions. The mosaic seeds had an average germination of 32 per cent as compared with 67 per cent in the case of seed from healthy plants. After germination the seeds were planted in 10-inch pots of sterilized soil and the plants were grown in a greenhouse which was isolated from those in which mosaic cucumbers were present. All mosaic plants in the other houses were kept under cages in order to avoid the danger of insect transmission of the disease. No aphids or striped cucumber beetles were found on the *Micrampelis* plants during any of the trials, and the houses were fumigated at intervals of 10 days to guard against their presence. The results of the trials are given in Table I.

It will be noted that in all the trials a certain number of the seedlings grown from seeds of mosaic wild cucumber plants developed the mosaic disease, while none of the control plants showed signs of it at any time. All of the mosaic plants showed symptoms of the disease soon after the seed germinated. In every case the symptoms were noticeable in the first leaf, and plants which did not show signs

TABLE I.—*Results of trials with seed from mosaic and healthy plants of Micrampelis lobata*

Date of planting	Number of plants	Condition of plants used as source of seed	Number of mosaic plants	Per cent of mosaic plants	Date observed
Apr. 9, 1919.....	110	Mosaic.....	13	11	Apr. 22, 1919
Apr. 5, 1919.....	200	Healthy (control).....	0	0	May 25, 1919
Apr. 12, 1920.....	174	Mosaic.....	10	5	Apr. 26, 1920
Apr. 15, 1920.....	219	Healthy (control).....	0	0	May 20, 1920
May 10, 1920.....	211	Mosaic.....	21	10	May 19, 1920
May 15, 1920.....	150	Healthy (control).....	0	0	June 8, 1920
Mar. 10, 1921.....	169	Mosaic.....	22	13	Mar. 20, 1921
Do.....	150	Healthy (control).....	0	0	Apr. 18, 1921
Mar. 12, 1922.....	253	Mosaic.....	18	7	Mar. 29, 1922
Mar. 10, 1922.....	387	Healthy (control).....	0	0	Apr. 21, 1922

Average infection from seed from mosaic plants, 9 per cent.

of the disease during this early period remained healthy throughout the experiment. This fact, together with the absence of mosaic on the control plants, seems to eliminate the possibility of infection from sources other than the seed.

Inoculations were made from a majority of the plants supposed to be mosaic to healthy cucumber plants and the actual presence of the disease was thus established. The early symptoms of mosaic on infected *Micrampelis* seedlings are often misleading, as they may frequently be confused with other abnormalities of growth which occasionally appear on plants of the wild cucumber. The first leaf of mosaic plants is usually wrinkled and somewhat mottled in appearance, but often the mottling is not sharply defined. Infected seedlings are always somewhat dwarfed, however, and the cotyledons are considerably smaller than those of healthy plants. The succeeding leaves in most cases are definitely mottled and have the curled and savoyed appearance which characterizes the mosaic disease on most cucurbits (pl. 1, B). In some instances, however, the leaves are a normal green, with the exception of minute yellow areas scattered irregularly over the surface. Such plants are somewhat dwarfed, but the leaves are regular in outline and show none of the wrinkling and curling mentioned above. In the field, where the plants have been attacked by insects or exposed to other unfavorable conditions, they often develop a similar appearance which is easily confused with mosaic. The later growth of mosaic seedlings, however, always shows typical mosaic symptoms.

The consistent appearance of mosaic on a portion of the seedlings grown from seeds of mosaic *Micrampelis*, together with the fact that the disease did not develop on the remaining plants during their later growth or on plants grown from healthy seed, seems to warrant the conclusion that the seed of the wild cucumber acts as a carrier of the disease. This has been further substantiated by the field observations described below.

DEVELOPMENT OF MOSAIC ON WILD CUCUMBER SEEDLINGS IN THE FIELD

Before definite evidence of seed transmission of *Micrampelis* mosaic was obtained it was already known that the disease was of common occurrence on this host (8) and that mosaic plants could be found in the spring before cultivated cucurbits had been planted. Since

this latter fact confirmed the results of the seed trials, a series of observations was made each year at points where mosaic *Micrampelis* plants had originally been noted.

CAGING EXPERIMENTS

Early in the spring of 1919 large cheesecloth cages were constructed over two spots where mosaic wild cucumbers had been found during 1917 and 1918. The cages covered a space of 4 by 6 feet and were 3 feet high, with the lower edge sunk 6 inches in the ground. The first cage was put down on April 10 before any of the *Micrampelis* seed had germinated, and the second was set out on May 1 over seedlings that had just appeared. No striped beetles or other cucumber insects had appeared at this time and the seedlings showed no evidence of insect injury. The cages were watched carefully in order to repair any breaks in the cloth and, as far as could be determined, no insects gained entrance during the experiment. On May 28 the plants in the cages were examined, and in the first cage 2 out of 11 plants showed definite mosaic symptoms. In the cage put down on May 1, 4 out of 26 plants were mosaic diseased. No insects were found in the cage and the plants showed no insect injury. It having been shown that the soil is not a carrier of the disease, these results offered further indication that the seed of *Micrampelis* acts as a carrier of cucurbit mosaic.

FIELD OBSERVATIONS

Various centers of mosaic wild cucumber plants found between 1917 and 1919 have since been kept under observation in order to determine the exact period at which the disease appears in the spring. These observations, begun in 1918 (8), have given abundant evidence that the disease develops in the same spot each season and that its appearance may be noted on the plants while in the seedling stages. The results, as shown in Table II, include observations at 12 of these points, in all of which the disease was found during successive seasons.

TABLE II.—Record of appearance of mosaic on seedlings of *Micrampelis lobata* at same points during successive seasons

Plant group	Locality	Period of observations					
		May 1 to June 1, 1919		May 1 to June 1, 1920		May 1 to June 1, 1921	
		Number of plants	Number of mosaic plants	Number of plants	Number of mosaic plants	Number of plants	Number of mosaic plants
I	Madison, Wis.....	68	9	12	2	0	-----
II	do.....	90	15	41	9	49	6
III	do.....	108	9	72	11	32	7
IV	do.....	40	7	30	4	33	6
V	do.....	15	3	15	2	34	5
VI	do.....	29	3	21	5	39	5
VII	do.....	-----	(b)	625	60	300	18
VIII	do.....	-----	(b)	28	3	76	9
IX	Portage, Wis.....	-----	(b)	350	25	250	34
X	Marengo, Ill.....	-----	-----	125	8	89	11
XI	do.....	-----	-----	35	7	19	4
XII	do.....	-----	-----	10	2	28	4
Per cent of plants mosaic.....		-----	13	-----	10	-----	11

I Mosaic plants also found during May, 1918.

II Mosaic plants found during July and August.

The wild cucumber seedlings in the vicinity of Madison, Wis., usually appear in the field between May 1 and May 15, and practically all the observations were made within two weeks after the development of the first leaf. All the plants which developed mosaic showed definite symptoms of the disease in the first leaves, and in many cases the presence of the disease was confirmed by inoculations from suspected plants to healthy cucumbers in the greenhouse. The possibility of infection from other sources was slight, as cultivated cucurbits are rarely planted at the time these observations were made, and near-by groups of healthy *Micrampelis* plants remained mosaic-free each season. The experiments previously mentioned, in which mosaic *Micrampelis* plants appeared in spots which had been caged early in the spring, also seem to reduce the possibility of the infection coming from any source except the seed. As will be shown later in this paper, the milkweed, *Asclepias syriaca*, is also an agency in overwintering cucurbit mosaic, but as the mosaic milkweed shoots do not appear until some time after the disease is found on the wild cucumber seedlings, the infection on *Micrampelis* could hardly come from this source. It is also of interest to note that the percentage of mosaic plants found in the open each year is approximately the same as that obtained in trials with seed from mosaic wild cucumber plants in the greenhouse. All the evidence, therefore, seems to warrant the conclusion that cucurbit mosaic is carried in the seed of mosaic plants of the wild cucumber.

It is a peculiar fact that cucurbit mosaic should be carried in the seed of the wild cucumber but not in that of the cultivated cucurbits. This anomaly is as yet unexplained, but Butler (3), in a review of the literature on mosaic diseases, makes an interesting suggestion. He points out that the embryo is not connected with the vascular system, as the bundles do not penetrate the nucellus, and states that—

In the Cucurbitaceae the nucellus is provided on the outside of its epidermis with a well-marked cuticle, and this epidermis persists in the ripe seed, though the rest of the nucellus is absorbed. The stalk end of the ovule (the chalaza) is suberified. Thus the whole of the central part of the ovule is cut off from the seed coats and stalk by cutin or suberin. The embryo also, in the spherical stage, is provided with a complete investment of cuticle over its free parts. All the evidence available in regard to infection by the virus diseases indicates strongly that they are unable to pass through cutin and probably unable to pass through suberin (e. g., uninjured branches or a potato tuber). Hence one would expect that the virus of cucumber mosaic would fail to be carried over to the next generation in the seed. But this is one of the diseases which is sometimes transmitted by seed. The explanation of this anomaly is perhaps to be found in the work of Longo (15), who has shown that since the cucurbit embryo is so completely isolated by impermeable layers, an adaptation of a curious nature has been developed, in which the pollen tube opens a communication between the neck of the nucellus and the embryo-sac. Possibly the same channel could serve to transmit the virus to the embryo.

INSECT TRANSMISSION OF MOSAIC FROM THE WILD TO THE CULTIVATED CUCUMBER

Mosaic wild cucumber plants often occur at considerable distances from fields of cultivated cucurbits, and these and other wild host plants would be of relatively minor importance were it not for the fact that insects serve as carriers of the disease. Experiments have demonstrated, however, that the insects which transmit the disease in the case of the cucumber (8) also transmit mosaic from *Micrampelis* to the cultivated cucurbits.

TRANSMISSION OF MOSAIC FROM THE WILD TO THE CULTIVATED CUCUMBER
BY THE CUCUMBER APHIS

In these experiments, a number of cucumber aphids, *Aphis gossypii* Glover, were colonized on mosaic and healthy wild cucumber plants. The aphids from mosaic plants were later transferred to healthy cucumber plants under cages, and the aphids from healthy *Micrampelis* plants were placed on healthy cucumbers in other cages as controls. As shown in Table III, infection occurred on all plants to which aphids from mosaic wild cucumber plants were transferred, while the controls remained healthy in each experiment.

TABLE III.—Transmission of cucurbit mosaic from *Micrampelis lobata* to healthy cucumber plants by *Aphis gossypii*

Date	Condition of plants used as source of aphids	Number of plants	Number of mosaic plants	Date observed
July 14, 1919.....	Mosaic.....	4	4	July 23, 1919
Do.....	Healthy (control).....	3	0	Do.
Aug. 18, 1919.....	Mosaic.....	7	7	Aug. 30, 1919
Do.....	Healthy (control).....	3	0	Do.
July 10, 1920.....	Mosaic.....	8	8	July 21, 1920
Do.....	Healthy (control).....	8	0	Do.
July 21, 1920.....	Mosaic.....	6	6	Aug. 2, 1920
Do.....	Healthy (control).....	6	0	Do.
Aug. 9, 1920.....	Mosaic.....	6	6	Aug. 20, 1920
Do.....	Healthy (control).....	6	0	Do.

TRANSMISSION OF MOSAIC FROM THE WILD TO THE CULTIVATED CUCUMBER
BY THE STRIPED BEETLE AND BY THE 12-SPOTTED BEETLE

The transmission of mosaic from the wild to the cultivated cucumber by the striped beetle, *Diabrotica vittata*, and by the 12-spotted beetle, *D. 12-punctata*, has been demonstrated by a number of experiments with both insects. The two species of *Diabrotica* are so similar in habit, however, that definite evidence of transmission by either species would indicate that both were concerned in carrying the disease.

In these experiments, beetles were collected at points supposedly distant from any sources of cucurbit mosaic infection, and then placed in cages containing healthy cucumber plants and allowed to feed for three days. The beetles were then removed and the plants were kept under observation for two weeks to determine whether any of the insects were carrying the mosaic virus when collected. In all cases, however, the test plants remained healthy. After the beetles had been tested in this manner, they were placed in cages containing healthy cucumber plants in 4-inch pots, together with a few potted plants of mosaic *Micrampelis*. The *Micrampelis* plants were so placed as not to be in contact with the cucumbers, thus insuring that no infection took place through contact of the leaves. The beetles were allowed to feed indiscriminately on both the cucumber and *Micrampelis* plants for periods ranging from three to seven days, and were then removed from the cages. Mosaic *Micrampelis* and healthy cucumber plants were placed in the control cages, but no insects were admitted. The results given in Tables IV and V indicate that the disease is readily transmissible by these insects under the conditions of these experiments.

TABLE IV.—*Transmission of cucurbit mosaic from Micrampelis lobata to healthy cucumber plants by Diabrotica vittata*

Date	Cage	Source of infection	Number of beetles in cage	Number of cucumber plants in cage	Number of mosaic plants	Date observed
May 6, 1919.....	1	1 mosaic <i>Micrampelis</i> plant in cage with healthy cucumbers.	25	6	3	May 15, 1919
Do.....	2	do.....	20	6	0	Do.
June 14, 1919.....	1	3 mosaic <i>Micrampelis</i> plants in cage with healthy cucumbers.	30	5	2	June 30, 1919
Do.....	2	do.....	20	5	0	Do.
June 11, 1920.....	1	do.....	20	8	3	June 30, 1920
Do.....	2	do.....	20	8	2	Do.
Do.....	3	do.....	20	8	1	Do.
Do.....	4	do.....	20	10	0	Do.

* Control.

TABLE V.—*Transmission of cucurbit mosaic from Micrampelis lobata to healthy cucumber plants by Diabrotica 12-punctata*

Date	Cage	Source of infection	Number of beetles in cage	Number of cucumber plants in cage	Number of mosaic plants	Date observed
July 10, 1919.....	1	3 mosaic <i>Micrampelis</i> plants in cage with healthy cucumbers.	25	6	2	July 25 1919
Do.....	2	do.....	25	6	3	Do.
Do.....	3	do.....	20	6	0	Do.
Aug. 15, 1920.....	1	1 mosaic <i>Micrampelis</i> plant in cage with healthy cucumbers.	30	6	3	Aug. 29, 1920
Do.....	2	do.....	25	4	1	Do.
Do.....	3	do.....	38	10	3	Do.
Do.....	4	do.....	20	8	0	Do.

* Control.

Although the beetles used were first tested by allowing them to feed on healthy plants, there is a remote possibility that they may have carried infection from outside sources. This seems unlikely, however, as the experiments with *Diabrotica vittata* were conducted early in the spring at a time when no cultivated cucurbits had appeared in the vicinity, so that the only probable source of infection would have been mosaic plants on which the beetles had fed before they were collected. Other experiments, however, were in progress at the same time, in which beetles collected in the open were fed on healthy cucumber plants, but no infection occurred in such cases. The experiments with *Diabrotica 12-punctata* were necessarily conducted at a later date, as this species does not appear in any considerable numbers until after cucumbers in the field have passed the seedling stage. No mosaic was found on cucumbers, however, until some time after the insects had been collected, so that mosaic wild cucumber plants were, in all probability, the only source from which the insects may have carried infection. The preliminary tests with these insects, however, seem to have eliminated this possibility.

IMPORTANCE OF THE STRIPED CUCUMBER BEETLE IN THE TRANSMISSION OF MOSAIC FROM THE WILD CUCUMBER

The striped cucumber beetle is apparently of greater importance than either the 12-spotted beetle or the cucumber aphid in carrying

the disease from the wild cucumber to cultivated cucurbits. This is due to the fact that this insect emerges from hibernation each year at approximately the time at which the wild cucumber seedlings appear. Observations during the last five years have shown that the beetles may often be found feeding on the cotyledons of *Micrampelis lobata* when the first leaf is still only partially developed. The striped beetle occurs in considerable numbers in most cucumber-growing sections and is usually found on the wild cucumber throughout May and June. The beetles apparently feed on the wild cucumber and other host plants until the cultivated cucurbits come up, and the majority then appear to migrate to the cultivated hosts. This migration from the wild to the cultivated cucurbits probably continues to a small extent throughout the season. When wild cucumbers have been grown within a few yards of cultivated cucumbers, a constant flight from one host to the other has frequently been noted and, as will be shown later, it is probable that considerable distances may be covered between the wild and cultivated plants.

The lesser importance of the 12-spotted beetles in this relation is due to the fact that they are not as frequently found on *Micrampelis* in May and June, when compared with the numbers of *Diabrotica vittata*, as the 12-spotted beetle does not appear in any considerable numbers in the vicinity of Madison, Wis., until about July 1. On this account much of the experimental work has been done with *Diabrotica vittata*. There is no reason to believe that the 12-spotted beetle is not an agency in the dissemination of mosaic from the wild cucumber later in the season, and to some extent at an earlier date as well. In any case, the results with one species seem directly applicable to the other, if both species are present at the same time.

The cucumber aphid, *Aphis gossypii*, is probably not of much importance in carrying infection from the wild cucumber over any considerable distance. The aphids are rarely found on *Micrampelis* until after mosaic has appeared on cucumbers in the field and are not of common occurrence on the wild host, so that while they are almost certain to produce infection if they move from mosaic *Micrampelis* to the cucumber, their actual importance is limited. The winged generations are the only ones likely to cover any great distance and their migrations are apparently limited when compared to those of the striped beetle. In view of the greater numbers and activity of the beetles it is probable, therefore, that the aphids are of minor importance in the dissemination of the disease from the wild cucumber.

INFECTION OF CUCUMBER PLANTS BY STRIPED BEETLES COLLECTED ABOUT MOSAIC MICRAMPELIS PLANTS IN THE OPEN

The rôle of the striped beetle as a means of transmitting primary infection from the wild cucumber to the cultivated cucurbits has been demonstrated to some extent by experiments in which the beetles were collected from groups of *Micrampelis* plants containing mosaic individuals and placed in cages containing healthy cucumber plants. No effort was made to collect the beetles from mosaic plants alone and the insects were taken from all plants in the vicinity. The results of these experiments, as shown in Table VI, indicate that beetles from such mosaic centers were able to transmit the disease in a number of cases.

TABLE VI.—*Transmission of cucurbit mosaic to healthy cucumber plants by striped beetles collected from groups of mosaic Micrampelis plants*

Date	Number of beetles in cage	Number of plants in cage	Number of plants mosaic	Date observed	Date	Number of beetles in cage	Number of plants in cage	Number of plants mosaic	Date observed
June 15, 1919	74	16	3	July 1, 1919	May 28, 1920	39	12	0	June 14, 1920
Do	57	20	5	Do.	June 7, 1920	41	12	0	June 18, 1920
June 18, 1919	40	10	2	Do.	Do.	52	15	2	Do.
Do	61	12	0	Do.	June 14, 1920	35	10	0	June 29, 1920
June 20, 1919	25	9	1	July 5, 1919	Do	35	10	0	Do.
June 29, 1919	40	12	0	July 12, 1919	Do	35	10	3	Do.
May 28, 1920	50	14	2	June 14, 1920					

The infection which occurred in 7 out of the 13 cages represents a fairly high percentage of infection in the case of the striped beetle, since many trials with beetles taken directly from mosaic cucumber plants have shown that relatively few such insects will transmit the disease. A considerable number of insects were placed in each cage, as the percentage of infection with the individual insects had always been low. This is in marked contrast to the results with the cucumber aphid, *Aphis gossypii*; but, as stated in an earlier paper (8), it is probably due partly to the fact that the beetle, a chewing insect, often attacks the stems and blossoms of the plant and, in a majority of cases, probably does not produce infection in wounds at these points. The results of artificial inoculation have shown that relatively little infection takes place through superficial injuries in the blossoms or on the surface of the stem.

DISTANCES OVER WHICH MOSAIC MAY BE TRANSMITTED FROM MOSAIC MICRAMPELIS PLANTS BY CUCUMBER BEETLES OR OTHER INSECTS

Although mosaic *Micrampelis* plants are a possible source of primary infection to cucurbits in the field, their importance in this connection rests to a great extent on the distances over which insects may transmit the disease from such plants. As already noted, the striped beetle, and to a lesser extent the 12-spotted beetle, seem the most important agents in such transmission, for they probably migrate more commonly and to greater distances than do aphids. An effort has been made to determine experimentally the distance which the disease may be transmitted by insects, in the belief that such transmission would most probably be through the agency of the cucumber beetle. Such experiments were necessarily only indicative, but were undertaken in the hope of accumulating some actual data on the spread of mosaic about a single group of mosaic wild cucumber plants.

An effort was made to remove all known or suspected host plants of cucurbit mosaic over a certain area, with the exception of a single group of mosaic *Micrampelis* plants which was left as a source of infection. Small plats of cucumbers were planted at varying distances from these wild cucumber plants, and under such conditions any mosaic occurring on the cucumbers could be traced with some certainty to the group of mosaic wild cucumbers. The area used for these experiments was nearly all under cultivation and almost free from known hosts of cucurbit mosaic. It extended north from

the mosaic *Micrampelis* for one-quarter mile to the shore of Lake Mendota, and one-half mile east and west. This area was carefully inspected to insure the removal of wild host plants, and it was believed any infection which occurred in the plats must be carried over a distance at least equal to that which separated them from the known source of mosaic.

These experiments were conducted on the same plan during 1920 and 1921. The number of plats was reduced in 1921, but those remaining occupied approximately the same positions as in 1920. Frequent inspections were made to note the first appearance of mosaic and as soon as it appeared in a plat all of the plants, both mosaic and healthy, were at once removed, except in one case which will be noted later. This was done to prevent the disease from spreading from plat to plat. The various dates of infection in the several plats are shown in Table VII.

TABLE VII.—Results of experiments to determine distances over which mosaic infection may be carried by insects from *Micrampelis lobata* to cucumber

1920				1921			
Plat	Dis- tances from mosaic <i>Micram- pelis</i>	Date beetles appeared in plat	Date mosaic appeared in plat	Plat	Dis- tances from mosaic <i>Micram- pelis</i>	Date beetles appeared in plat	Date mosaic ap- peared in plat
	<i>Yds.</i>				<i>Yds.</i>		
1a ^a -----	1	June 11	June 27	1a-----	1	June 8	June 21
2-----	140	do-----	July 16	2b-----	175	do-----	Aug. 28
3b-----	175	do-----	Aug. 10	3c-----	225	do-----	July 17
4c-----	225	do-----	Aug. 2	4d-----	350	June 10	Aug. 18
5d-----	350	do-----	July 23	5e-----	500	do-----	June 30
6-----	350	do-----	Aug. 2				
7e-----	500	do-----	July 28				

^a Letters indicate plats in approximately same location in 1920 and 1921; 1920 plats planted May 28 1920; 1921 plats planted May 28, 1921.

In both years many striped and a few 12-spotted beetles were already present when the seed was planted and the insects appeared in all the plats while the plants were still in the seedling stage. In both 1920 and 1921 the plants in plat 1, which was directly adjacent to the mosaic wild cucumbers, were infected at an earlier date than plants in any of the other plats. Observations showed that there were frequent migrations of beetles from the wild cucumbers to this plat. In both seasons there was an irregularity of infection as related to the distance from the supposed source of mosaic infection on *Micrampelis*. This is noticeable in plats 2 and 3 of the 1921 experiment, which were in an open field at a distance of approximately 50 yards from one another. Infection in plat 3 occurred on July 17, while in plat 2, which was nearer the wild cucumbers, the disease did not develop until August 28. The same fact is even more evident in the most distant plats of the two experiments, in both of which the disease developed much earlier on plants at a distance of 500 yards from the mosaic *Micrampelis* plants than in plats at half that distance. In the case of part 5, in the experiments of 1921, the infection recorded on June 30 consisted of a single plant, which was removed when first observed. As there was practically no possibility

of this infection being carried by those making the observations, and as no further infection appeared until late in August, it is quite probable that the original infection came from a distant source, such as the mosaic wild-cucumber plants, and that, owing to the chance nature of infection from beetles over such distances, no further infection occurred for some time.

Perhaps such an irregularity in infection is to be expected if we consider the beetles to have been the agency concerned in the transmission of the disease, for, as already stated, the percentage of infection due to these insects is always low as compared with that due to aphids. On this basis a certain element of chance may exist, and it is possible that a greater number of insects might visit a nearby plat without necessarily causing immediate infection, while a smaller number might reach a more distant plat and produce infection at an earlier date. In most cases it is improbable that great numbers of insects feed on *Micrampelis* and immediately travel to the cucumbers in the field. Recent investigations by Dudley, of the United States Bureau of Entomology (12) indicate that the beetles may travel as far as one-half mile and also that they perhaps pass over nearby cucumber fields when moving with the wind. Under such circumstances the above results probably are to be expected.

If we accept the results of these experiments at their face value, they indicate the possibility of infection being carried at least a quarter of a mile from groups of mosaic *Micrampelis* plants and at a date sufficiently early to allow for destructive development of the disease. In the plats within 350 yards of the *Micrampelis* plants, it was practically certain that infection must have been carried a distance equal to that of the known source on the wild cucumber. We can not be sure, however, that insects other than the cucumber beetles were not concerned in its transmission. This latter point is of minor significance, however, as regards the practical importance of the wild cucumber as a source of primary infection.

The source of infection for the most distant plats (plat 7 of 1920 and plat 5 of 1921), is less certain, perhaps, especially as the disease developed comparatively early at this point each year. These plats occupied approximately the same locations and were close to a small piece of wooded land which contained many wild plants. Careful inspection at various times failed to show any known host plants of cucurbit mosaic, but the possibility of their being overlooked is not beyond question. The general evidence, however, seems to indicate that infection was traceable to the wild cucumber. In theory, the distance over which the beetles may carry infection is limited only by the extreme range of their flight. Field observations, however, indicate that a range of 400 to 600 yards represents the limits within which there is a reasonable expectation of infection on the cultivated hosts, since at these distances fields have often remained free from mosaic for the greater part of the summer.

IMPORTANCE OF THE WILD CUCUMBER AS A SOURCE OF INFECTION OF THE CULTIVATED CUCURBITS

The present evidence has shown that the mosaic disease is carried over winter in the seed of the wild cucumber and that certain insects are able to transmit the disease from this host to the cultivated

cucurbits. The actual importance of the wild cucumber as a source of primary infection to the cultivated hosts is dependent, however, on the numbers of mosaic *Micrampelis* plants which occur in a given locality and their location with respect to fields of cultivated cucurbits. The factor of the distances over which insects carry the infection is, of course, equally important, but, for the present at least, it is assumed that this distance is approximately 400 to 500 yards. In most localities the striped beetle is always present in considerable numbers, so the presence of an insect carrier is assured.

SURVEY OF 1919

If the wild cucumber is actually an important factor in overwintering mosaic, infected wild plants would be expected to occur in considerable numbers in districts where the mosaic disease is severe in the fields. Surveys of the past three years have shown that a distinct correlation of this sort exists in Wisconsin and others of the Central States, although the surveys have been chiefly confined to Wisconsin.

In these surveys all of the fields of cucumbers and other cucurbits in a given area were inspected for the presence of mosaic infection and an effort was made to locate as many groups of wild cucumber plants in the vicinity as possible. The occurrence of mosaic *Micrampelis* plants in different localities and the corresponding occurrence of mosaic on other cucurbits is shown in Table VIII. Aside from a few localities, however, no attempt was made to locate all of the mosaic wild cucumber plants in a given area. While the presence of the disease was noted, no attempt was made to show the number of cucumber fields visited or the number of these fields which were mosaic, because in sections visited late in the season there was naturally a greater amount of mosaic present than in localities visited when the disease had first appeared, as it had had an opportunity to spread from field to field during the intervening period. Under these circumstances the wild cucumber as a source of infection bears only slight relation to the extent of mosaic in the field, because only the early infection can safely be attributed to this source. The average number of fields visited in all localities was approximately 12, and in localities where the disease occurred mosaic was present in about 40 per cent of the fields.

The results of these surveys emphasized the fact that the wild cucumber occurs abundantly throughout the Central States. In many localities large groups of these plants occur along streams and in other moist situations and are found in smaller numbers in low places along roads, railway embankments, fence hedgerows, and other similar spots. The distribution of the plant has been greatly increased by the fact that it is often used as an ornamental, so that large numbers of wild cucumber plants are found about dwellings in most towns and villages. It is frequently found in neglected spots about farm buildings as a result of having been introduced as an ornamental, and a thorough survey in some localities has revealed many more plants than would be noted by a cursory inspection.

TABLE VIII.—Occurrence of mosaic on *Micrampelis lobata* in various localities as related to the presence of the disease on cucumbers in the vicinity

Date	Locality	Square miles of area covered	Number of centers of Micrampeils	Number of mosaic centers	Occurrence of mosaic on cucumbers in area
<i>Survey of 1919</i>					
July 9.....	Sturgeon Bay, Wis.....	1.5	7	0	0
July 10.....	Ellison Bay, Wis.....	1.0	2	0	0
Do.....	Green Bay, Wis.....	2.5	11	0	0
July 25.....	do.....	1.0	6	0	0
July 24.....	Menominee, Mich.....	2.0	13	0	0
Do.....	Marinette, Wis.....	1.5	11	0	0
July 11.....	Racine, Wis.....	4.0	19	4	(a)
July 18.....	Madison, Wis.....	5.0	24	10	(b)
July 22.....	Milwaukee, Wis.....	1.5	6	0	(a)
Aug. 30.....	do.....	2.0	8	2	(a)
July 26.....	Portage, Wis.....	3.0	8	3	(a)
Aug. 28.....	Ripon, Wis.....	2.0	27	11	(b)
Aug. 29.....	Princeton, Wis.....	2.0	30	19	(b)
Do.....	Fond du Lac, Wis.....	0.5	5	1	(a)
<i>Survey of 1920</i>					
June 11.....	Sparta, Wis.....	1.0	8	5	(b)
July 24.....	Harvard, Ill.....	2.0	18	8	(b)
July 26.....	Marengo, Ill.....	1.5	11	5	(a)
Aug. 24.....	Huntington, Long Island.....	(c)	1	1	(a)
Aug. 25.....	Riverhead, Long Island.....	2.0	3	1	(a)
Aug. 26.....	Southampton, Long Island.....	(c)	1	1	(a)

* Indicates presence of mosaic.

b Unusually severe mosaic infection.

c No definite area covered.

As shown in Table VIII, no mosaic was found on cucumbers in the district around and north of Green Bay, in northwestern Wisconsin. There is no record of the disease in this section, although cucumbers are grown on a commercial scale in most of the localities visited. No mosaic was found on any wild cucumber plants in this part of the State. On the other hand, a survey over much of the cucumber-growing territory in the southern portion of Wisconsin revealed the more or less frequent occurrence of mosaic on the wild cucumber correlated with its presence on the cultivated cucurbits in the vicinity.

In many localities the disease was found on a considerable number of *Micrampelis* plants, while in other places it occurred on but few. As closely as could be estimated from observations of this type, there was a very striking correlation between the number of groups of mosaic plants of the wild cucumber and the extent of the disease in the fields, although, as previously stated, it was impossible to draw conclusions too definitely from observations made in various localities over a period of only six to seven weeks. It will be seen from Table VIII, however, that the disease was found on a number of wild cucumber plants at Madison, Ripon, and Princeton, Wis., during 1919, and that the disease was unusually severe in the fields at these points. Ripon and Princeton are of particular interest from the fact that the disease is known to have occurred in these localities as early as 1900, the earliest record of its appearance in Wisconsin. It has always been particularly severe in both places. The survey

of 1919 brought out the fact there were hundreds of mosaic wild cucumber plants in both towns and along the streams in the neighborhood. The number of mosaic centers and the total number of such plants found at both Ripon and Princeton was far in excess of that at most other points visited.

As considerable data were available on the previous losses from mosaic at most points visited, it was possible by this means to determine to some extent the probable relation of *Micrampelis* to infection in the field. In localities such as Ripon, Princeton, Madison, and Sparta in Wisconsin, and Harvard and Marengo in Illinois, where mosaic has long been a serious problem, there were considerable numbers of mosaic wild cucumber plants. In most of the other sections where there were fewer mosaic wild cucumbers, the disease has apparently been severe only in certain seasons; although some infection occurs each year.

The probable importance of the wild cucumber in overwintering mosaic was also emphasized by the fact long noted that the mosaic disease is more severe on cultivated cucumbers in the immediate vicinity of towns and villages than in outlying districts. This is probably due in part to the proximity of the fields to one another and the consequent ease of dissemination of the disease and in part to the fact that mosaic wild cucumbers are much more common about towns than in the country by reason of the greater use of the *Micrampelis* vine as an ornamental in and near the towns. It was also evident that mosaic on the cucumber was much more common in districts where there were numerous small streams and low places, which are the natural habitat of *Micrampelis lobata*.

Reports from other sections indicate that the wild host is common in all of the Central and Eastern States. Where surveys have been made in those States in sections in which the disease occurs, the presence of mosaic wild cucumbers has usually been established. The writers therefore consider that they are justified in regarding *Micrampelis lobata* as an important source of mosaic infection in most regions where cucurbits are grown on a commercial scale.

Recent investigations have shown that the milkweed (*Asclepias syriaca*) and the pokeweed (*Phytolacca decandra*) are also concerned in the overwintering of cucurbit mosaic and that they are frequently a source of primary infection to the cucurbits. Because of these other agencies by which the disease is carried over winter, the writers have not been able to definitely determine the comparative importance of a single host in this group, because their actual importance varies with the locality. It is evident, nevertheless, that the wild cucumber plays an important part in overwintering cucurbit mosaic.

MILKWEED AS AN AGENCY IN OVERWINTERING CUCURBIT MOSAIC

The existence of mosaic diseases on certain wild plants has been known for some time, but until 1920 there was no evidence that such diseases could be transmitted to the cucurbits. Jagger (14) had successfully transmitted cucumber mosaic to single species of the Compositae and Lobeliaceae, and one of the writers of this paper (Doolittle) had also brought about infection on *Martynia louisiana* Mill. (8), but aside from indicating that the disease was transmissible

to plants of other families than the Cucurbitaceae these results had little bearing on the problem of overwintering. More recent investigations, however, have shown that a number of plants of families outside the cucurbits are susceptible to cucurbit mosaic, and among these the milkweed, *Asclepias syriaca*, is an important agency in the overwintering of the disease.

FIELD OBSERVATIONS

The occurrence of mosaic on the milkweed was noted in 1916 by E. A. Bessey, of the Michigan Agricultural Experiment Station, who sent specimen plants to the senior writer. Inoculations were made to cucumber plants with this material, but no infection resulted. Milkweed plants which appeared to be mosaic were also found during 1917 and 1919, and a number of inoculations were made to the cucumber; but, prior to 1920 (8), there was no evidence, as far as the writers are aware, that milkweed mosaic was transmissible to the cucurbits. The studies with the wild cucumber, however, led indirectly to a further interest in the problem of the relation of the milkweed to the overwintering of mosaic.

AT MADISON, WIS.

During the summer of 1919, an effort was made to locate the source of primary infection in a field known as the Olin plat at Madison, Wis., which had been used for the experiments on the overwintering of mosaic in the soil. This plat was isolated and at a considerable distance from other cucumber fields, but it was noted that mosaic appeared there earlier and in more severe form than at any other point in the vicinity.

Several careful inspections failed to show the presence of mosaic wild cucumber plants within a mile of this point, although in most other mosaic-diseased fields about Madison it was possible to find wild cucumber plants sufficiently near to account for the infection. The early appearance of the disease on the Olin plat added to the peculiar aspect of the problem, and consequently all wild plants in the vicinity were examined for evidences of mosaic. It was found that the plat itself contained approximately 20 milkweed plants which showed marked symptoms of a disease similar to cucurbit mosaic. A further investigation of milkweeds in the neighborhood failed to show any other mosaic plants, and this fact added to the importance of the discovery, for it indicated a possible relation between the diseases on the two hosts. Such a possibility was of particular importance, as the milkweed is perennial and could therefore carry the disease over winter if susceptible.

The Olin plat was again planted to cucumbers in 1920, and record was made of the mosaic milkweed plants which appeared there. Approximately 20 such plants were found between the rows of cucumbers up to July 12, in addition to a considerable number of healthy plants. The first infection on the cucumber was noted July 12, and the field was thereafter inspected at intervals of three to four days. As the disease developed, record was made of the location of each mosaic plant and the proximity of mosaic milkweeds. These observations showed that practically all of the early infection on the cucumber had occurred within 6 to 10 feet of mosaic milkweeds, as shown by the accompanying field plan (fig. 1).

Aside from these plants, there was practically no mosaic infection on the cucumbers in this plat until about July 25. The cucumber aphid (*Aphis gossypii*) was present in considerable numbers from July 1 to the end of the season, and as a result 75 per cent of the cucumber plants became mosaic diseased between August 1 and August 15. The aphids were present on the mosaic milkweeds before the disease developed on the cucumber plants, and their presence on the milkweed supported the theory that this plant might be a source of primary infection to the cucumber, as the aphids were known to be carriers of the disease. If this were the case, the cucumbers directly adjacent to the mosaic milkweeds would probably be the first infected, for aphids ordinarily do not travel very far. As a result of these observations, it seemed probable that the milkweed might be of considerable importance in relation to cucurbit mosaic.

AT ROCKLAND, WIS.

Soon after the above observations were made, further evidence

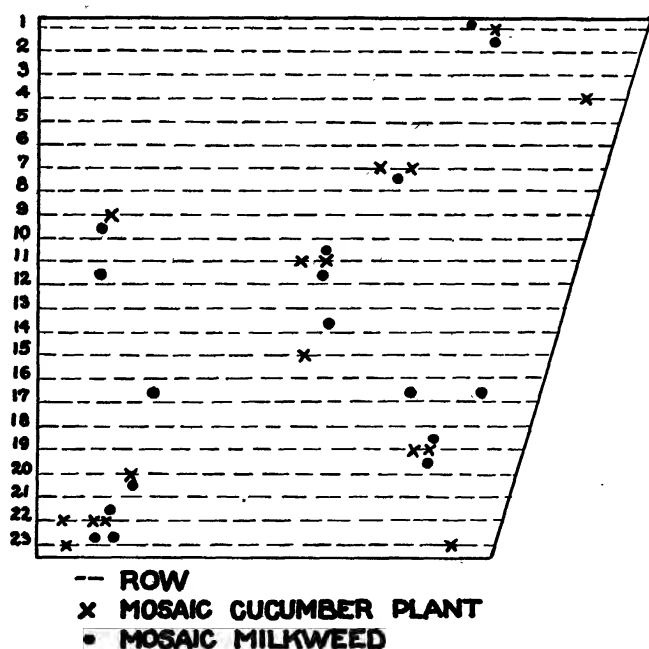


FIG. 1.—Plan of experimental plat at Madison, Wis., showing occurrence of first mosaic infection on cucumber plants directly adjacent to mosaic milkweeds. July 12 to 19, 1920

was obtained at Rockland, Wis., which eventually established the milkweed as a source of mosaic infection for the cultivated cucurbits. During 1920, experiments in regard to the possible control of cucurbit mosaic through the removal of wild cucumber plants were conducted at Rockland and all mosaic *Micrampelis* plants which could be found in the vicinity were removed during June and early July. Inspections were made about every two weeks and no mosaic was found on cucumbers in the vicinity until August 5. On this date one of the

fields in the area from which wild cucumbers had supposedly been removed was found to be 90 per cent mosaic diseased. As only two other fields in the vicinity showed any trace of mosaic and as the infection was comparatively slight in both cases, the source of infection in the badly diseased field furnished a peculiar problem. A brief investigation, however, showed that nearly 100 mosaic milkweed plants were growing between the rows of cucumbers and that both the cucumbers and milkweeds were heavily infested with the cucumber aphid. Further inspection revealed the presence of mosaic milkweed plants in another of the less severely infected fields, and in this case also aphids were found on both hosts. The evidence at Rockland and Madison was so striking that cross-inoculation experiments were immediately begun in a further attempt to determine the possible identity of the mosaic diseases occurring on the two hosts.

CROSS-INOCULATIONS FROM MOSAIC MILKWEED TO THE CUCUMBER
 INOCULATION WITH APHIDS

Aphids from mosaic milkweeds found in the fields at Rockland were brought to Madison and transferred to healthy cucumber plants under cages in the greenhouse. Definite cases of mosaic occurred on all these plants, and the controls on which aphids from healthy milkweeds had been placed remained free from mosaic. These results were followed by a series of experiments in which aphids from healthy cucumbers were transferred to mosaic milkweed plants which were free from insects. These plants were covered with cheesecloth cages, and after the aphids had been allowed to feed on them for some time a number of the insects were transferred to healthy cucumber plants under cages in the greenhouse. This method eliminated the possibility that mosaic infection produced by aphids from mosaic milkweeds in the field might be due to aphids which had recently migrated from mosaic cucumber plants to the milkweed. The results given in Table IX show that nearly all cucumber plants inoculated in this manner developed typical cases of mosaic.

TABLE IX.—*Results of cross-inoculation from mosaic milkweeds (Asclepias syriaca) to healthy cucumber plants by means of Aphis gossypii*

Date, 1920	Source of inoculum	Method of inoculation	Number of plants inoculated	Number of mosaic plants	Date observed, 1920
Aug. 5.....	Rockland, Wis..	Aphids from mosaic milkweed.....	9	9	Aug. 12
Do.....	do.....	Aphids from healthy milkweed (control) ..	9	0	Aug. 18
Aug. 15.....	Madison, Wis..	Aphids from mosaic milkweed *.....	7	6	Aug. 27
Do.....	do.....	Aphids from healthy milkweed (control) ..	7	0	Sept. 1
Aug. 16.....	do.....	Aphids from mosaic milkweed *.....	6	5	Aug. 27
Do.....	do.....	Aphids from healthy milkweed (control) ..	6	0	Sept. 1
Sept. 1.....	do.....	Aphids from mosaic milkweed *.....	8	8	Sept. 19
Do.....	do.....	Aphids from healthy milkweed (control) ..	8	0	Do.
Sept. 4.....	do.....	Aphids from mosaic milkweed *.....	4	3	Sept. 13
Do.....	do.....	Aphids from healthy milkweed (control) ..	4	0	Do.
Sept. 5.....	do.....	Aphids from mosaic milkweed *.....	9	9	Sept. 20
Do.....	do.....	Aphids from healthy milkweed (control) ..	5	0	Sept. 23

* Aphids from healthy cucumber plants transferred to mosaic milkweed plants under cages in the field.

ARTIFICIAL INOCULATION

In addition to the cross-inoculation by means of *Aphis gossypii*, the susceptibility of the cucumber to the mosaic disease on the milkweed was confirmed by artificial cross inoculations. The crushed leaf tissues of mosaic milkweeds were used as inocula and a small fragment of the crushed leaf was inserted in a slight longitudinal cut in the lower portion of the stem of healthy cucumber plants. The oldest leaf of the inoculated plant was cut off close to the stem and the incision made in this wounded surface. The controls were treated in the same manner, except that the crushed leaf tissue of healthy milkweed plants was inserted in the stem. Inoculations were made both in the field and in the greenhouse and all plants inoculated were kept under cheesecloth cages to avoid outside infection. The results of these experiments, shown in Table X, have been uniformly successful, and the fact of the transmission of the mosaic disease from the milkweed to the cucumber is thus established.

TABLE X.—*Results of artificial cross inoculation of healthy cucumber plants with crushed leaf tissue of mosaic milkweeds*

Date (1920)	Source of inoculum	Location of inoculated plants	Number of plants inoculated	Number of mosaic plants	Date observed, 1920
Aug. 6.....	Rockland, Wis.....	Greenhouse.....	8	3	Aug. 12
Do.....	do.....	do. ^a	8	0	Aug. 18
Do.....	do.....	Field.....	12	8	Aug. 12
Do.....	do.....	do. ^a	7	0	Aug. 18
Aug. 12.....	Madison, Wis.....	Greenhouse.....	7	7	Sept. 1
Do.....	do.....	do. ^a	5	0	Do.
Aug. 13.....	do.....	Field.....	7	4	Do.
Do.....	do.....	do. ^a	9	0	Do.
Aug. 16.....	do.....	Greenhouse.....	6	4	Do.
Do.....	do.....	do. ^a	4	0	Do.
Do.....	do.....	Field.....	8	6	Do.
Do.....	do.....	do. ^a	7	0	Do.
Sept. 1.....	do.....	Greenhouse.....	8	5	Sept. 19
Do.....	do.....	do. ^a	8	0	Do.

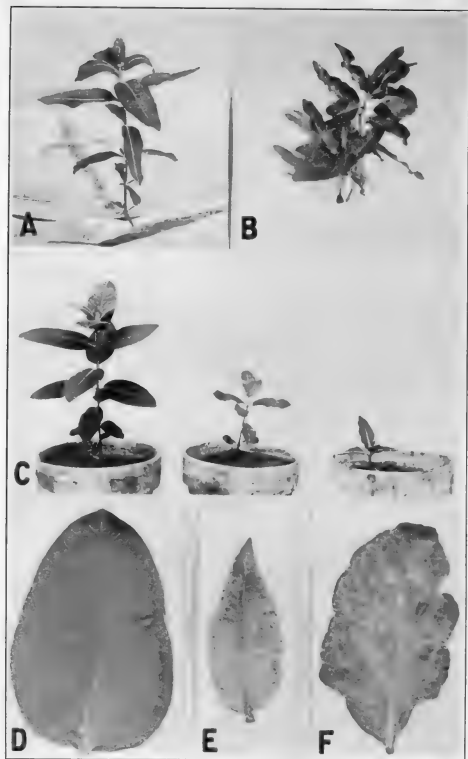
^a Control.

CROSS INOCULATIONS FROM MOSAIC CUCUMBER PLANTS TO THE MILKWEED

The identity of the mosaic disease of the milkweed with that occurring on the cucurbits has also been shown by the results of inoculations from mosaic cucumber plants to healthy milkweeds. These inoculations have been relatively limited in number, but the results have been so definite as to amply demonstrate the possibility of such infection.

During the summer of 1920 cages were placed over four healthy milkweed plants, and aphids from a mosaic cucumber plant were colonized on each of them. Aphids from healthy cucumber plants were placed on four other healthy milkweeds as controls. These inoculations were made on August 12, and on September 1 the inoculated plants were found to show a curling and mottling of the leaves similar to the symptoms of mosaic milkweeds in the field. The controls, however, also showed a less severe curling of the leaves, although there was no change in color. In order to make sure that the apparent mosaic symptoms were not merely aphid injury, the aphids were removed from both the inoculated and control plants on September 4. The plants were then allowed to remain until September 29, when they were again examined. On this date the new growth of the inoculated plants showed evident symptoms of mosaic, while the later growth of the control plants was normal in character. The presence of mosaic was proved in the case of the inoculated plants by the transference of aphids from these plants to healthy cucumbers. Between September 2 and September 4, aphids from all the inoculated and control plants were transferred to healthy cucumbers under separate cages in the greenhouse. As shown in Table XI, the aphids from all of the inoculated milkweeds produced mosaic on the cucumbers, while the plants to which the aphids from control milkweeds were transferred remained normal. The success of these inoculations was further demonstrated by experiments herein described, in which the roots of these inoculated plants were grown in the greenhouse (pl. 2, B, C).

Further successful cross inoculations from mosaic cucumbers to the milkweed were made in experiments in the greenhouse during 1920-21. Five healthy milkweeds, grown from roots brought in



MOSAIC MILKWEED LEAVES AND PLANTS

A. Mosaic milkweed plant, *Asclepias syriaca*, found in cucumber plats at Madison, Wis., showing slight mottling and deformation of the leaves. Inoculations from this plant produced mosaic on healthy cucumber plants. Madison, Wis., August, 1922

B. Mosaic milkweed plant inoculated by means of aphids from a mosaic cucumber plant, showing extreme type of mosaic symptoms. Madison, Wis., August, 1920

C. Mosaic milkweed plants (at right) grown in greenhouse from roots of mosaic plant shown in B. Healthy plant (at left) grown from roots of healthy plant collected in the field Madison, Wis., February, 1920.

D. Young leaf of healthy milkweed plant

E, F. Young leaves of mosaic milkweed collected at Rockland, Wis., July, 1922

from the field, were placed in cages and inoculated by means of aphids taken from mosaic cucumber plants. Healthy cucumber aphids were also placed on three other healthy milkweed plants as controls. The inoculations were made on February 4, and on February 24 all the aphids present on the plants were removed by spraying with nicotine sulphate. Aphids from healthy cucumber plants were transferred to both the inoculated and control milkweeds on March 1, and on March 7 some of the insects from each set of milkweeds were transferred to healthy cucumber plants under separate cages. The results of these inoculations, given in Table XI, show that infection occurred on the cucumbers on which aphids from the inoculated milkweeds were placed, while the aphids from the controls produced no infection.

TABLE XI.—*Results of cross inoculations of healthy cucumber plants from milkweed plants inoculated with cucurbit mosaic by means of aphids*

MILKWEED PLANTS INOCULATED IN FIELD, AUG. 13, 1920

Date of inoculation	Milkweed used as inoculum	Method of inoculation	Number of cucumber plants inoculated	Number of mosaic plants	Date observed
Sept. 1, 1920	1-1	Aphids	4	4	Sept. 13, 1920
Do	• 23-1	do	4	0	Do.
Do	1-1	Crushed leaf tissue	4	3	Do.
Do	• 23-1	do	4	0	Do.
Sept. 4, 1920	1-1	do	11	7	Sept. 15, 1920
Do	• 1-4	do	10	0	Do.
Do	7-1	Aphids	4	3	Do.
Do	• 1-4	do	4	0	Do.
Sept. 10, 1920	17-1	do	4	4	Sept. 19, 1920
Do	• 23-2	do	4	0	Do.
Do	17-1	do	4	2	Do.
Do	• 23-2	do	4	0	Do.
Sept. 12, 1920	17-1	do	5	5	Do.
Do	• 22-1	do	5	0	Do.
Do	17-2	do	5	5	Do.
Do	• 22-2	do	5	0	Do.

MILKWEED PLANTS INOCULATED IN GREENHOUSE, MAR. 20, 1921

Apr. 11, 1921	A-1	Crushed leaf tissue	8	3	Apr. 25, 1921
Do	A-3	do	8	4	Do.
Do	A-4	do	8	5	Do.
Do	A-5	do	8	0	Do.
Do	A-6	do	8	3	Do.
Do	• A-8	do	8	0	Do.
Do	• A-9	do	8	0	Do.

• Control plant.

The results of direct inoculation from the cucumber to the milkweed have been less uniformly successful, as only 2 definite cases of mosaic have been produced out of 15 plants inoculated in the field and greenhouse. These inoculations were made by inserting the crushed leaf tissue of a mosaic cucumber plant into longitudinal incisions at various points in the stems of healthy milkweeds and also by pricking the juices of mosaic cucumber plants into the younger leaves. The excessive flow of latex which follows the slightest wounding of the milkweed may account in part for the difficulty experienced in infecting by this method.

SYMPTOMS OF MOSAIC ON MILKWEED

The symptoms of the mosaic disease on the milkweed are similar in character to those found on the cucurbits. Mosaic plants are easily recognized by their dwarfed growth and mottled and distorted leaves (pl. 2, A, B). Mosaic milkweeds rarely reach a height of more than 2 feet, while normal plants usually attain 3 to 4 feet. Mosaic leaves are ordinarily mottled with irregular patches of greenish yellow. These areas are larger proportionately than in the case of the cucumber, and the mottled appearance is ordinarily more marked in character. This mottled character varies, however, with the individual plants and is occasionally much less pronounced (pl. 2, D, E, F).

The leaves of mosaic milkweeds are distorted in shape to an extent which is uncommon in the cucurbits, and the symptoms in this regard are more suggestive of the mosaic disease on tobacco. The leaves are ordinarily smaller than those of the normal milkweed and usually are more lanceolate than the oblong to ovate leaves of healthy plants. In some instances the leaves are of abnormal length and taper rapidly from a broad base to an almost filiform tip. Very commonly half of the leaf blade develops to almost normal size while the other half is only a few millimeters wide for all or part of its length. This effect of the disease produces abnormalities which are readily recognized. The leaves of mosaic plants also show a tendency to curl upward at the margins, producing a cuplike effect in extreme cases (pl. 2, B).

The dwarfed character of the plants, many of which are not more than 12 inches high, accompanied by the characteristic symptoms on the leaves, make the mosaic disease easily recognizable in the field. It has been found, however, that normal milkweed plants frequently show a mottling of the leaves which may be confused with the true mosaic. Such plants are of normal height, however, and show none of the curling or distortion characteristic of mosaic. The mottling in such cases is also different from that produced by mosaic in that it usually consists of areas which are limited by the parallel lateral veins and extends from the midrib to the edge of the leaf. These areas produce a striped effect, while in the case of mosaic, the yellowish areas are less regular in outline and are scattered irregularly over the leaf. Symptoms similar to those described above have also been observed on a single plant of *Asclepias amplexicaulis* Sm., but no infection was secured from inoculations made from this plant to the cucumber.

OVERWINTERING OF THE MOSAIC DISEASE IN THE MILKWEED

EXPERIMENTS WITH THE ROOTS OF MOSAIC MILKWEED

Since mosaic shoots are produced each year from the roots of other mosaic perennials such as the pokeweed (*Phytolacca decandra*) (2), it was expected that the mosaic disease would be carried from year to year in the roots of the milkweed. Observations in the Olin plat at Madison, Wis., during 1919 and 1920 showed that mosaic milkweeds appeared in the summer of 1920 at approximately the same points in which they had been noted during the preceding year. Experimental proof of such overwintering was obtained during the winter of 1920-21 in the case of roots of milkweeds brought in from the field and grown in the greenhouse. A number of mosaic and

healthy milkweed plants in the Olin plat were marked during the early fall, and in November a portion of the roots of these plants was dug and placed in 10-inch pots in the greenhouse. In most cases the roots of mosaic and healthy plants were grown in separate pots, but in two instances mosaic and healthy roots were planted together.

The roots in practically every instance sent up from one to two shoots during the early part of February and it was possible to determine the presence of mosaic on such shoots. From the results given in Table XII, which includes all such plants, it will be seen that the roots of mosaic plants produced shoots which were mosaic diseased in every case (pl. 2, C). The roots of the supposedly healthy plants, however, developed healthy shoots in all instances. In the two pots in which both healthy and mosaic roots were placed, both healthy and mosaic shoots were produced.

TABLE XII.—Results obtained from growing roots of healthy and mosaic milkweed plants in the greenhouse at Madison, Wis., and inoculating healthy cucumbers from the plants thus produced

Roots (brought in on Nov. 10, 1920)	Character of roots	Manner of in- fection	Type of shoots produced	Number of cucum- ber plants inoculated from shoots	Method of inocu- lation	Number of plants mosaic
1-1-----	Mosaic-----	Inoculated-----	Mosaic-----	4	Aphids-----	3
7-1-----	do-----	do-----	do-----	4	do-----	2
8-1-----	do-----	Natural-----	do-----	10	do-----	8
8-2-----	do-----	do-----	do-----		None-----	
9-1-----	do-----	do-----	do-----		do-----	
9-2-----	do-----	do-----	do-----	3	Aphids-----	1
12-1-----	do-----	do-----	do-----	2	do-----	0
12-2-----	do-----	do-----	do-----	3	do-----	2
13-1-----	do-----	Inoculated-----	do-----	9	Aphids and arti- ficial-----	8
13-2-----	do-----	do-----	do-----	7	do-----	3
14-2-----	do-----	Natural-----	do-----		None-----	
17-1-----	do-----	Inoculated-----	do-----	4	Artificial-----	3
17-2-----	do-----	do-----	do-----		None-----	
19-1-----	do-----	Natural-----	do-----	2	Aphids-----	1
19-2-----	do-----	do-----	do-----	2	do-----	2
9-H2 ^a -----	Healthy-----		Healthy-----	4	do-----	0
10-1 ^a -----	do-----		do-----	4	do-----	0
10-2 ^a -----	do-----		do-----	6	Artificial-----	0
11-1 ^a -----	do-----		do-----		None-----	
15-2-----	do-----		do-----		do-----	
15-3-----	do-----		do-----	4	Aphids-----	0
A-1-----	do-----		do-----		None-----	
A-3-----	do-----		do-----		do-----	
A-4-----	do-----		do-----		do-----	
A-5-----	do-----		do-----		do-----	
A-6-----	do-----		do-----		do-----	
A-7-----	do-----		do-----	8	Aphids-----	0
A-8-----	do-----		do-----	8	do-----	0
A-9-----	do-----		do-----	8	do-----	0
A-10-----	do-----		do-----		None-----	

^aRoots of plants used as controls for plants indicated as "Inoculated" in Table XI.

These results, as shown in Table XII, were confirmed by a considerable number of inoculations from both the healthy and mosaic milkweeds to healthy cucumber plants. Inoculations in this case were made successfully both by means of aphids and by artificial inoculation with the crushed leaf tissues of the milkweed. It was also found that the roots of milkweeds inoculated with cucurbit mosaic in the field produced mosaic shoots in every case, while the roots of the controls of these experiments produced only healthy shoots.

These experiments demonstrate that milkweed plants when once infected with mosaic will produce mosaic shoots during succeeding seasons. Field observations during 1921 supported this belief, as mosaic milkweed plants were found during July, 1921, at all points where they occurred in 1920. These observations included the Olin plat at Madison, Wis., three fields at Rockland, Wis., and two at Marengo, Ill. In all of these fields the mosaic milkweed plants were found in the same spots during both 1920 and 1921, while at Madison the record also included 1919. In addition to these data, 12 mosaic milkweed plants were marked in the plat at Madison during the fall of 1920. Observations during June and July, 1921, showed that shoots of mosaic milkweed plants had appeared within 6 feet of nine of the points marked. In the other three instances no plants were seen during the season.

TRIALS WITH SEED FROM MOSAIC MILKWEEDS

As already mentioned, mosaic milkweeds rarely produce seed. In a few cases, however, seed has been obtained from them, and experiments have been conducted to determine whether the mosaic disease is carried over winter in the seed as well as in the roots of mosaic plants. The seed from mosaic plants was grown in flats of sterilized soil in a greenhouse in which the temperature was held at approximately 28° C. Seed from healthy plants was grown in other flats in the same house as a control. Up to the present, 387 seedlings have been grown from the seed of mosaic milkweed plants, but no evidence of mosaic has been noted on any of these plants, many of which were grown until they had reached a considerable size. These results, therefore, indicate that the disease is not carried in the seed of mosaic milkweed plants.

IMPORTANCE OF THE MILKWEED AS A SOURCE OF CUCURBIT INFECTION

In view of the evidence just given, it is apparent that the milkweed is susceptible to cucurbit mosaic, and that the perennial rootstalks of mosaic milkweed plants carry the disease over the winter. As with the wild cucumber, however, the importance of the milkweed as a source of infection rests on the nature of the carriers which transmit the disease to the cultivated cucurbits, and upon the time of appearance, number, and location of the mosaic milkweeds.

INSECT CARRIERS OF MILKWEED MOSAIC

APHIDS

The cucumber aphid (*Aphis gossypii*), as shown in this paper, is known to be an agent in transmitting mosaic from the milkweed to the cucumber. These aphids are commonly found on milkweed plants in the vicinity of cucumber fields infested with the insects, but are comparatively rare on milkweeds at a distance from cultivated cucurbits. Other species of aphids are often found on the milkweed, but up to the present no other species has been found which will feed on the cucumber. It seems probable, therefore, that in most cases the cucumber aphids reach the milkweed from infested cucurbits in the vicinity.

DIABROTICA BEETLES

Preliminary experiments with the striped and 12-spotted cucumber beetles, *Diabrotica vittata* and *D. 12-punctata*, indicate that these insects do not feed as readily on the milkweed as do the cucumber aphids. Both species of beetles, however, are found on the milkweed to a slight extent during the early summer, when cucurbits are not available as food. When caged with milkweed plants, both species attacked the plants to a certain extent, although the 12-spotted beetles feed more readily on the milkweed than the striped species. When striped beetles have been placed in cages containing mosaic milkweed and healthy cucumber plants, the cucumber plants in a few cases have become mosaic diseased, indicating that the beetle is capable of transmitting mosaic from the milkweed to the cucumber. The evidence to date is rather too meager to justify definite conclusions, but it seems probable that the beetles may occasionally carry infection from the milkweed to the cucumber. It is doubtful, however, whether they are of much importance as compared with the aphids as carriers of the disease from this host. No insects other than those mentioned above have been studied in relation to the transmission of milkweed mosaic.

OCCURRENCE OF MOSAIC MILKWEEDS IN THE FIELD

As the cucumber aphid seems to be the chief agency by which mosaic is transmitted from the milkweed to the cucurbits, it is probable that the mosaic milkweeds which occur in the immediate neighborhood of the fields are the only ones of importance as sources of primary infection. This belief is further strengthened by the fact that most cucumber aphids found on the milkweed have originally come from adjacent cucumbers and that these insects are comparatively limited in their flight.

Field observations during 1921 showed that mosaic milkweeds are frequently found in close proximity to fields of cucumbers, and also that such plants are rarely found in more distant locations. The field studies have been made principally at Madison and Rockland, Wis., and at Marengo, Ill. The surveys in these localities were confined to areas of from 1 to 3 square miles in which there were a number of cucumber fields. All the milkweeds which could be found in these areas during June and July were examined for evidences of mosaic. Later surveys were also made in the neighborhood of cucumber fields which developed mosaic.

At Madison only four groups of mosaic milkweed plants were found. The largest of these, already mentioned as occurring in the Olin plat, consisted of 27 plants, all of which were between rows of cucumbers. Approximately 100 milkweeds within 50 yards of the plat were examined, but no mosaic plants were found outside the cucumber plat. Four mosaic milkweed plants were found between the rows of cucumbers in another plat about a mile from the Olin field, and in this case also all milkweeds outside of the plat seemed free from the disease. Two other small groups of milkweeds showing mosaic were found in gardens in the neighborhood, both of which contained mosaic cucumber plants. Approximately 300 other milkweeds examined along roadsides and in open fields in the vicinity were all found to be healthy, so that up to the present the only mosaic milkweeds found at Madison have been in fields planted to cucurbits for several seasons.

At Rockland the fact that mosaic milkweeds were found near cucumber fields only was emphasized by surveys during 1921 and 1922. Over an area of 2 square miles, which included 10 cucumber fields, an effort was made to locate as many milkweed plants as possible and some hundreds of plants were examined during both seasons. Only two groups of mosaic plants were found at a distance of more than a few yards from cucumber fields. On the other hand, seven groups of mosaic milkweeds were found growing either in and immediately adjacent to fields of cucumbers or on land which had grown cucumbers in 1920. Two of these groups were at the same points where mosaic plants were found in 1920. The mosaic milkweeds at the different points varied in number from 3 to 100, and most of them were found between June 10 and July 20. In three instances the mosaic milkweeds were between rows of cucumbers. In four of the other six groups they were on land where mosaic cucumbers had grown in 1920. In all cases the mosaic plants were within 50 yards of cucumber fields, and as aphids were present during the latter part of July there was ample opportunity for the transmission of the disease to the cucumbers. It was not possible to trace the first mosaic infection on the cucumber to the milkweed as directly as in 1920, but in one field the first mosaic cucumber plants were found growing about a mosaic milkweed plant which had appeared late in the season.

At Marengo only five groups of mosaic milkweed plants were found during the season of 1921. The area covered was much larger than at Rockland, and the cucumber fields were scattered and at considerable distances from one another. Four of the five groups of mosaic milkweed plants were found on land which had grown cucumbers in 1920. Mosaic milkweeds were found in this field in 1920 and infection also occurred on the cucumbers during that year. In two of the four cucumber fields in which mosaic milkweeds were found in 1921, infection appeared three weeks earlier than in any of the remaining nine fields in the area examined. In every case where mosaic milkweeds were found the land had grown several successive crops of cucurbits.

At both Rockland and Marengo mosaic milkweed plants were found before cultivated cucurbits had appeared, so that this source of infection was present from the beginning of the season. Most of the mosaic milkweeds appeared during June and early July, but in some cases mosaic plants were found just breaking ground during the latter part of August. Aside from obtaining the data just referred to, little survey work has been done with regard to milkweed mosaic. Mosaic milkweeds were found in 1920 in three cucumber fields on Long Island, N. Y., in which nearly all of the cucumber plants were affected with mosaic. A further inspection of fields of other crops showed a large number of milkweeds in the vicinity, but no mosaic milkweeds were found at such points. Mosaic milkweed plants were also noted during 1921 at Sparta and Portage, Wis., and at Harvard, Ill.

It is evident from these observations that mosaic milkweeds, while comparatively rare, occur in most cases in the immediate vicinity of cucumber fields, or on land which has previously grown cucurbits. In view of the fact that practically no mosaic is found on milkweed plants in isolated situations or on farms where cucurbits are not

grown, it seems probable that the milkweed in most cases is first infected from the cucumber. This infection probably occurs chiefly through the agency of aphids and the mosaic persists in the milkweeds from year to year and thus furnishes a source of primary infection to nearby cucurbits.

COMPARATIVE IMPORTANCE OF THE MILKWEED AND WILD CUCUMBER IN OVERWINTERING CUCURBIT MOSAIC

When the milkweed was first discovered to be a factor in overwintering cucurbit mosaic, it was thought that it was probably of minor importance in comparison with the wild cucumber. Further investigations, however, have indicated that mosaic milkweeds are a serious menace to the cucurbits, and, as far as opportunity for infection is concerned, they are often of more importance than the wild cucumber. This belief is based on field observations made in the same localities for three seasons and is further substantiated by the results of experimental studies.

Mosaic wild cucumbers in the majority of cases occur at distances of from 100 to 500 yards or more from cucumber fields, except in the vicinity of towns, where they occasionally are very near the fields and are very numerous. In the case of this host, the *Diabrotica* beetles appear to be the chief means of disseminating the disease, as aphids are not commonly found on *Micrampelis* plants and do not cover as great distances as the beetles. It has also been found that only a relatively small percentage of the beetles which feed on mosaic wild cucumber plants are likely to transmit the disease to cultivated cucurbits. The field experiments previously described have also shown that at distances of 100 to 500 yards the infection of cucurbits from mosaic *Micrampelis* plants is of irregular occurrence. The whole question of infection from the wild cucumber depends on the factor of the transmission of the mosaic disease by the *Diabrotica* beetles, and the probability of the transmission of the disease from this host is apparently less than in the case of the milkweed.

The number of mosaic milkweed plants appears to be small when compared to the number of wild cucumber plants which have been found in the same localities, but in most cases the milkweeds occur either within or near the cucumber fields. Aphids are probably the most important means of transmitting the disease from the milkweed to the cucumber, and these insects will readily travel the distances which usually separate the mosaic milkweed plants from the cucumbers. Moreover, the aphids are practically certain to produce infection if transferred to healthy plants, while the *Diabrotica* beetles appear to transmit the disease only in a certain percentage of cases after feeding on mosaic plants. Although the situation varies with the locality, it is probable that the few mosaic milkweeds which do occur near the cultivated cucurbits are of more potential importance than a comparatively larger number of mosaic *Micrampelis* plants which are scattered over a wide area.

OVERWINTERING OF CUCURBIT MOSAIC ON THE POKEWEED

The presence of a mosaic disease on another perennial, the pokeweed (*Phytolacca decandra*), has been known for some time, but until recently there was no evidence that it was transmissible to plants of other families. Woods (19), in 1902, reported the occurrence of a

mosaic disease on this host, and Clinton (4), in 1915, stated that he had been unable to transmit pokeweed mosaic to healthy tobacco plants. Allard (1), in 1914, also mentioned pokeweed mosaic and reported that it was not transmissible to tobacco. In a later paper (2), Allard showed that the disease was readily transmissible to healthy pokeweed plants, but stated that he was unable to transmit it to tobacco or pepper. One of the writers (Doolittle) (8) also reported that he had been unable to transmit pokeweed mosaic to the cucumber. All of the inoculation experiments mentioned above were made by artificial methods, using the expressed juices or crushed tissues of mosaic plants.

The possibility of a relationship between the mosaic disease of pokeweed and that occurring on the cucurbits was first indicated by observations made by W. W. Gilbert at Holland, Mich., in 1915, where mosaic pokeweeds were found in abundance near a field of mosaic cucumbers. The first successful cross inoculations from the pokeweed to the cucumber were obtained in the course of experiments with the cucumber aphid.⁴ Aphids from mosaic cucumber plants had been colonized on certain plants of species supposedly insusceptible to cucurbit mosaic, in order to determine whether the infective principle of the disease was transmissible to the offspring of aphids which had fed on mosaic plants. Among the plants used were a few pokeweeds, and it was noted that the majority of these plants showed symptoms of a mosaic disease within two weeks after the aphids had been placed on them. As a result of this evidence, further cross-inoculation experiments were undertaken, in which aphids were used as a means of inoculation.

METHODS OF INOCULATION

As already indicated by the experiments with the milkweed, the use of aphids as a means of inoculation has been the most important factor in demonstrating the wide host range of the disease. In the earlier studies of cucurbit mosaic (8) it was found that inoculations in which aphids were transferred from mosaic to healthy cucumber plants resulted in nearly 100 per cent infection, but that a considerably lower percentage of infection was obtained when plants were artificially inoculated with the crushed tissues or expressed juices of mosaic plants. The advantages of insects as a means of inoculation became more apparent in the cross-inoculation experiments described in this paper.

In the case of the milkweed, pokeweed, and martynia, earlier cross inoculation by artificial methods (8) had given only negative results, and it was supposed that these plants were not susceptible to cucurbit mosaic. The use of aphids as a means of inoculation, however, demonstrated later that cucurbit mosaic could be transmitted to all of these hosts and to several other plants of widely separated families. While infection has since been produced on many of these hosts by artificial methods of inoculation, the first infection has always resulted from insect inoculations and the results have been much more consistent than those obtained by the artificial method.

⁴ The studies of the mosaic disease of pokeweed were conducted by the junior writer of this paper and presented as a master's thesis at the University of Wisconsin.

When aphids were used as a means of inoculation they were colonized on healthy cucumber plants under cages in the greenhouse or field. Healthy aphids from this stock were later transferred to the plant to be inoculated. The insects were ordinarily transferred by means of a camel's hair brush, and the number placed on each plant varied from 10 to 60. Ordinarily 10 to 15 aphids are found sufficient to insure infection, but in the earlier experiments with the pokeweed it was found that only a small percentage of the aphids remained on the plants after their transfer and the number used was therefore increased.

INFECTIOUS NATURE OF POKEWEED MOSAIC

As already shown by Allard (2), pokeweed mosaic is readily transmitted to healthy plants of this species by artificial inoculation. In order to be certain that the plants supposedly infected by means of the cucumber aphid were actually mosaic diseased, a number of inoculations were made from these plants to healthy pokeweeds, both by means of insects and by artificial methods. The results given in Table XIII show that the disease is readily transmissible to the pokeweed by either method of inoculation. The incubation period of the disease of the pokeweed, as in the case of that of the cucumber, varies to some extent with the rapidity of growth of the infected plant. The average incubation period appears to be from 5 to 10 days, and is therefore approximately identical with that of cucurbit mosaic.

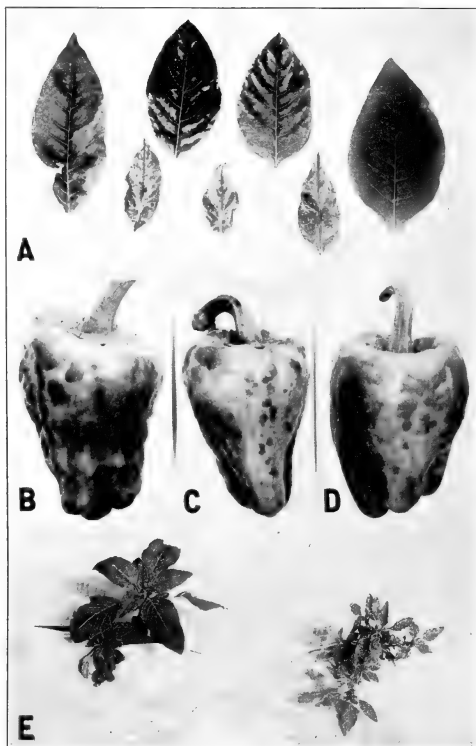
TABLE XIII.—Results of inoculations of healthy pokeweeds from pokeweeds infected with cucurbit mosaic

Date of inoculation	Source of inoculum	Number of plants inoculated	Number of plants mosaic	Date observed
APHID INOCULATION *				
1922				1922
Mar. 15.....	Aphids from mosaic pokeweed.....	9	6	Mar. 30
Mar. 22.....	do.....	4	2	Apr. 7
Mar. 31.....	do.....	1	1	Apr. 10
ARTIFICIAL INOCULATION				
Mar. 6.....	Crushed leaf tissue of mosaic pokeweed.....	6	2	Mar. 31
Mar. 22.....	do.....	8	8	Apr. 6
Do.....	Control.....	8	0	Do.
Mar. 23.....	Crushed leaf tissue of mosaic pokeweed.....	8	2	Apr. 11
Do.....	Control.....	8	0	Do.
Mar. 25.....	Crushed leaf tissue of mosaic pokeweed.....	3	1	Do.
Do.....	Control.....	3	0	Do.
Apr. 13.....	Crushed leaf tissue of mosaic pokeweed.....	24	20	Apr. 26
Do.....	Control.....	24	0	Do.
Apr. 24.....	Crushed leaf tissue of mosaic pokeweed.....	12	12	May 18
Do.....	Control.....	12	0	Do.

* Because of the fact that aphids had failed to colonize on the healthy plants intended for use as controls during these experiments, a number of healthy plants in the greenhouse were used as controls. None of the plants of this healthy stock developed mosaic.

SYMPTOMS OF MOSAIC ON THE POKEWEED

The symptoms of pokeweed mosaic are very similar to those of the mosaic diseases affecting the cucumber and tobacco. The disease first appears on the young leaves as a mottling of light greenish yellow in which the light areas are of small size. This mottling is accompanied by a downward curling of the midrib of the leaf, similar to



MOSAIC POKEWEEED LEAVES, FRUITS OF MOSAIC PEPPER, AND MOSAIC
PIGWEEED PLANT

A. Young leaves of mosaic pokeweed infected with cucurbit mosaic. Healthy pokeweed leaf at right. Madison, Wis., 1922

B, C, D. Fruits of mosaic pepper plant, *Capsicum annuum*, showing dark green wartlike areas on surface. Madison, Wis., 1920

E. Mosaic plant of the pigweed, *Amaranthus retroflexus*, showing mottling of foliage and dwarfed growth. Inoculations from this plant produced mosaic symptoms on healthy cucumber. Healthy pigweed plant at left. Madison, Wis., August, 1922

that found in mosaic cucumber plants. The light-green portions of the leaf may later include the greater portion of its surface. They are irregular in outline and do not appear to be delimited by the veins of the leaf (pl. 3, A). In the later stages of the disease the plants have a typically mosaic appearance, the darker portions of the leaf are slightly raised and the foliage has the wrinkled and blistered appearance which is associated with many mosaic diseases (pl. 4, A, C). The leaves of mosaic pokeweed plants are usually more irregular in outline than those of healthy plants. In the older plants the leaves are often curled upward at the edges in a manner similar to that of mosaic milkweeds.

Where plants are affected with the disease while young they become much stunted and blossom very sparingly. In the field, however, mosaic plants are commonly found which are 4 to 5 feet in height and appear to be making a vigorous growth. In all cases, however, the foliage shows the typical symptoms of the disease and the leaves are generally somewhat smaller than those of healthy plants.

SUSCEPTIBILITY OF THE POKEWEED TO CUCUMBER MOSAIC

As a result of the apparent infection of the pokeweed by aphids from mosaic cucumber plants, further cross inoculations were made from mosaic cucumber and muskmelon to the pokeweed, both by means of aphids and by artificial inoculation with the expressed juices of these plants. As shown by Table XIV, these experiments have shown that cucurbit mosaic is transmissible to the pokeweed by means of aphids from both the cucumber and muskmelon. Up to the present, however, no infection has been produced through wound inoculations with the expressed juices of these cucurbits. If only artificial methods of inoculation had been used the results of our earlier work, together with the results of Allard and Clinton in cross inoculation with tobacco, would have indicated that the pokeweed was not susceptible to other mosaic diseases and that the mosaic disease of pokeweed was distinct from that on the cucumber. It should be noted, however, that continued efforts at cross inoculation with cucurbit mosaic by artificial methods have eventually resulted in infection of host plants of other families, although earlier experiments had indicated that these hosts could not be infected except by means of aphids. It is possible, therefore, that the pokeweed may be infected with cucurbit mosaic by the use of artificial methods of inoculation. The use of the insect method is limited to some extent by the fact that it is not always possible to find insects that will transmit the disease in question and also feed on both the mosaic host plant and the one to be inoculated. The wide host range of the cucumber aphid, however, has made this insect of especial value in such experiments.

Some difficulty was encountered in the earlier experiments with the pokeweed, owing to the fact that during the winter the cucumber aphids often refused to feed on the pokeweed unless confined on the leaves in small cages. These cages were constructed by covering a Van Tieghem cell with fine wire screen and placing the cell over the leaf. The aphids were placed in the cage thus formed on the leaf and another piece of fine wire was placed on the under side of the leaf directly beneath the glass cell. The whole was then held in



MOSAIC POKEWEEED AND MUSKMELON PLANTS

- A. Mosaic pokeweed plants, *Phytolacca decandra* (at right) inoculated from a mosaic cucumber plant. Healthy control plants at left. Madison, Wis., April, 1923
- B. Mosaic muskmelon plants (at left) inoculated by means of aphids from a mosaic pokeweed plant. Healthy control plant at right. Madison, Wis., 1923
- C. Mosaic pokeweed plant (at right) inoculated with cucumber mosaic, showing extreme distortion and mottling of foliage. Healthy control plant at left. Madison, Wis., 1923

TABLE XIV.—Results of cross inoculations from mosaic cucumber to healthy pokeweed plants by means of aphids

Date of inoculation, 1922	Method of inoculation	Number of aphids per plant	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
Mar. 15.....	Aphids from mosaic cucumber plant.....	16	3	0	Apr. 6
Apr. 6.....	do.....	15	6	5	Apr. 14
Do.....	Aphids from healthy cucumber plant (control).....	15	2	0	Do.
Apr. 17.....	Aphids from mosaic cucumber plant.....	15	4	0	May 8
Do.....	Aphids from healthy cucumber plant (control).....	15	2	0	Do.
May 8.....	Aphids from mosaic cucumber plant.....	25	8	5	May 17
Do.....	Aphids from healthy cucumber plant (control).....	25	4	0	Do.
July 16.....	Aphids from mosaic cucumber plant.....	25	4	1	July 25
Do.....	Aphids from healthy cucumber plant (control).....	25	2	0	Do.
July 29.....	Aphids from mosaic cucumber plant.....	30	12	8	Aug. 7
Aug. 3.....	do.....	30	12	6	Aug. 11
Do.....	do.....	(a)	3	2	Aug. 9
Do.....	Aphids from healthy cucumber plant (control).....	25	12	0	Do.

* Aphids transferred naturally from mosaic cucumber plant in same cage.

place by a clamp, the screen on the under leaf surface preventing crushing by the clamp. This method proved successful, but after a time it was found that if a large number of aphids were colonized on the plants a few would remain without being confined in any way. It was also noted that the offspring of the aphids which remained on the pokeweed would feed more readily on this host than did the parent insects. The use of cages was therefore abandoned as unnecessary. It was later found that aphids would colonize readily on the pokeweed in large numbers during the spring and summer, possibly on account of some change in the juices of the pokeweed owing to its more vigorous and succulent growth.

The results of the inoculations by means of aphids were not as consistent with the pokeweed as with some other hosts, owing to the earlier difficulty in the colonization of the insects on the plants. The evidence, however, shows that the mosaic disease of cucurbits may be readily transmitted to the pokeweed.

TRANSMISSION OF MOSAIC FROM THE POKEWEED TO THE CUCURBITS

Reciprocal inoculations have also been made from mosaic pokeweed plants to the cucumber and muskmelon. In these inoculations the writers were again able to produce infection when aphids were used as a means of inoculation but were unsuccessful in producing infection by artificial inoculations. The results, as shown in Table XV, indicate that both the muskmelon and cucumber are susceptible to pokeweed mosaic (pl. 4, B). This being the case, it is evident that the pokeweed represents another perennial wild host of cucurbit mosaic.

OVERWINTERING OF CUCURBIT MOSAIC ON THE POKEWEED

TRIALS WITH ROOTS OF MOSAIC PLANTS

Since the pokeweed is a perennial and is known to be susceptible to cucurbit mosaic, it would be expected that the disease would live over winter in the roots of infected plants. Field observations in Michigan during 1915, 1916, and 1923, and at Anna, Ill., during 1922 and 1923, have shown that the roots of mosaic plants will again

TABLE XV.—*Results of cross inoculations from mosaic pokeweeds to healthy cucumber and muskmelon plants by means of aphids*

Date of inoculation, 1922	Method of inoculation	Number of aphids per plant	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
<i>Cucumber</i>					
Feb. 15.....	Aphids from mosaic pokeweed plant.....	25	1	1	Feb. 20
Feb. 24.....	do.....	10	4	0	Mar. 13
Do.....	Aphids from healthy pokeweed plant (control).....	10	2	0	Do.
Mar. 15.....	Aphids from mosaic pokeweed plant.....	15	2	0	Mar. 22
Do.....	Aphids from healthy pokeweed plant (control).....	15	2	0	Do.
Mar. 16.....	Aphids from mosaic pokeweed plant.....	15	2	1	Mar. 20
Mar. 20.....	do.....	15	10	9	Mar. 24
Do.....	Aphids from healthy pokeweed plant (control).....	15	4	0	Do.
July 10.....	Aphids from mosaic pokeweed plant.....	15	8	4	July 16
Do.....	Aphids from healthy pokeweed plant (control).....	15	4	0	Do.
Aug. 21.....	Aphids from mosaic pokeweed plant.....	(a)	49	25	Aug. 31
Do.....	Aphids from healthy pokeweed plant (control).....	(a)	28	0	Do.
Aug. 22.....	Aphids from mosaic pokeweed plant.....	15	12	11	Do.
Do.....	Aphids from healthy pokeweed plant (control).....	15	12	0	Do.
Aug. 24.....	Aphids from mosaic pokeweed plant.....	15	15	11	Do.
Do.....	Aphids from healthy pokeweed plant (control).....	15	12	0	Do.
<i>Muskmelon</i>					
Aug. 4.....	Aphids from mosaic pokeweed plant.....	30	3	3	Aug. 14
Do.....	Aphids from healthy pokeweed plant (control).....	30	3	0	Do.
Aug. 25.....	Aphids from mosaic pokeweed plant.....	15	12	9	Sept. 1
Do.....	Aphids from healthy pokeweed plant (control).....	15	12	0	Do.

* Aphids allowed to transfer naturally from mosaic and healthy pokeweed plants in the respective cages.

produce mosaic shoots during the following year. Roots from such plants were collected at Anna in 1923 and taken to Madison, Wis. In all cases they produced mosaic shoots, and infection was produced by inoculation from these plants to the cucumber. Pokeweeds inoculated from mosaic cucumber plants were planted in the open at Madison in the summer of 1922 and the plants were allowed to overwinter. Most of these plants were winterkilled, but the few which survived produced mosaic shoots during the spring of 1923. It has also been found that when the top of a mosaic plant is cut back to the roots the new shoots are always mosaic diseased. It appears, therefore, that the mosaic disease which affects the pokeweed in the field is transmissible to the cucurbits and that the disease is carried over winter in the roots of the mosaic plants.

TRIALS WITH SEED FROM MOSAIC POKEWEEDS

In addition to the trials with roots of mosaic plants, seed from mosaic pokeweeds has also been tested in order to determine whether the disease is carried over winter in this manner. A considerable amount of seed was collected for the writers by E. A. Bierbaum at Anna, Ill., during the summer of 1922, from both mosaic and healthy pokeweeds. As in the case of the trials with seed from the milkweed, the seed was planted in flats of sterilized soil in the greenhouse and grown at a temperature of approximately 28° C. Seed from healthy pokeweed plants was used as a control. These trials included about 800 seedlings from mosaic plants, but there was no evidence that the disease is carried in the seed. This evidence of nontransmission of pokeweed mosaic in the seed is in keeping with observations made by W. W. Gilbert at Donaldson, Ind., in 1922, and by the senior author at Anna, Ill., in 1923. At both points it was found that cucumber

fields near mosaic pokeweed plants contained a number of pokeweed seedlings, all of which were free from mosaic. Practically all of the old pokeweeds in the vicinity, however, were severely affected with mosaic.

INSECTS AS AGENCIES IN THE DISSEMINATION OF POKEWEED MOSAIC FROM THE
POKEWEED TO THE CUCURBITS

APHIDS

It was first thought that the cucumber aphids would feed on the pokeweed only when forced to do so by a lack of other host plants. This belief was at first confirmed by an inspection of mosaic pokeweeds in southern Illinois during the summer of 1922. No aphids or other insects were found on the pokeweed plants examined at that time, although they showed signs of insect injury at some earlier date. No aphids were found in the surrounding cucumber fields, however, so the evidence in this case was not conclusive. There was some question as to whether there was any common insect carrier of the disease from the pokeweed to the cucumber, and unless such a carrier existed it was not certain that the pokeweed was an important source of infection to the cucurbits. Later in the summer, however, it was found that the cucumber aphids were present in considerable numbers on pokeweeds in the experimental plats at Madison, and that their occurrence was not due to artificial conditions. The aphids had appeared naturally on the cucumbers and later had apparently migrated to the near-by pokeweed plants. The natural transference of the aphids from one host to the other was also demonstrated by experiments in which five mosaic pokeweed plants were placed in separate cages which also contained a number of healthy cucumber plants. The experiments were conducted in the field, and care was taken to see that the pokeweed and cucumber plants were not in contact. In each of these cages the mosaic pokeweeds soon became covered with aphids from the cucumbers, and in every case the cucumber plants in the cage became mosaic diseased within 12 days after the pokeweeds were introduced. In the control cages in which healthy pokeweeds had been placed there was no mosaic infection on the cucumbers in any case.

The migration of aphids from cucumbers to the pokeweed was also demonstrated by experiments in which healthy pokeweed plants were placed in three cages containing mosaic cucumber plants which were infested with aphids. In these experiments all of the pokeweeds became covered with the aphids, although they were not in contact with the cucumbers, and later developed symptoms of mosaic. These observations and experiments indicate that the cucumber aphid is a carrier of mosaic from the pokeweed to the cucurbits and that the aphids may also be responsible for a portion of the mosaic infection found on the pokeweed. The situation in the case of pokeweed seems much like that of the milkweed, although the evidence of the transmission of mosaic from the pokeweed to the cucurbits in the field is not as complete as that which exists with regard to the milkweed, owing to the fact that it has been necessary to conduct most of the work in Wisconsin, where the pokeweed is of rare occurrence in the field.

OTHER INSECTS

The only other insects which have been tested as possible carriers of pokeweed mosaic are the striped and 12-spotted *Diabrotica* beetles. In experimental trials with these insects the writers have found no evidence which indicates that they are agents in the transmission of mosaic from the pokeweed, but the work so far has been of a limited nature. Both insects have occasionally been found feeding on the pokeweed and are often found between the young leaves at the tip of the plants, although there is little evidence to indicate any extensive feeding on this host. At present the available evidence does not indicate that they are important factors in transmitting the disease to the cucurbits.

IMPORTANCE OF THE POKEWEED AS A SOURCE OF INFECTION TO THE CUCURBITS

In Wisconsin and northern Illinois the pokeweed is of little importance in overwintering cucurbit mosaic because of its rare occurrence. Up to the present the writers have never found pokeweed plants in the vicinity of cucumber fields in Wisconsin. In southwestern Michigan, however, mosaic pokeweeds have been observed in the vicinity of cucumber fields in Allegan, Berrien, Ottawa, and Van Buren Counties. The disease has been noted in abundance by W. W. Gilbert in the vicinity of Donaldson, Ind., where cucumber mosaic is also common. M. W. Gardner, of the Indiana Agricultural Experiment Station, reports that mosaic pokeweeds are abundant in the vicinity of melon fields at Vincennes, Ind., where cucurbit mosaic has been serious for some time. In the summers of 1922 and 1923 one of the writers visited fields of cucumbers at Anna, in the extreme southern portion of Illinois, which had suffered severely from mosaic for several seasons. Pokeweed plants were found to be numerous along the roadsides and in fence rows about the fields. At least 50 per cent of the plants observed were affected by mosaic, and in many cases they occurred within a few feet of cultivated cucurbits. As the pokeweed appears to be a potential source of infection to the cucurbits, the observations in the Anna district indicate that the opportunities are far greater for infection from this host than in any of the localities where the milkweed and wild cucumber are apparently responsible for overwintering mosaic.

The occurrence of such extensive infection on the pokeweed was of special interest, as neither the wild cucumber nor the milkweed seemed to occur in the locality. A careful search revealed only one group of plants of *Asclepias syriaca*, and these were at a distance of some miles from cucumber plantings and were free from mosaic. No wild cucumber plants were found in the vicinity and the local growers seemed unfamiliar with both the wild cucumber and milkweed. Gardner also reports that he has seldom seen either the wild cucumber or milkweed in southern Indiana. As these hosts are not common in the vicinity of Anna, it seems probable that the mosaic infection on the cucurbits is traceable to the mosaic pokeweeds in the vicinity of the fields. Further observations have made this theory seem more probable, since mosaic pokeweeds have been found growing in hotbeds which contained young cucumber plants ready for setting out in the field. These pokeweeds were so

large that they must have been present for at least three seasons prior to the observations. Under these conditions, the presence of aphids would be very likely to result in infection of the young cucumber plants and the disease would in this way be introduced early in the season. The field evidence, therefore, indicates that the pokeweed should be classed with the wild cucumber and milkweed as a source of primary infection in some localities.

PRESENT STATUS OF THE PROBLEM OF OVERWINTERING

From the work described above, it is evident that the importance of the various wild host plants varies with the locality. Up to the present, however, all of our observations in Wisconsin, Indiana, and Illinois have shown that mosaic infection is common on at least one of the above wild host plants in all districts where the disease is prevalent on the cultivated cucurbits. The overwintering of mosaic on the wild cucumber, milkweed, and pokeweed seems to be established, but it is possible that the list of host plants may be increased by further investigations.

Recent experiments have confirmed the work of Muncie (16), which indicates that the catnip, *Nepeta cataria* L., may also be a perennial host of cucurbit mosaic. The importance of this host in the field in Wisconsin has not yet been determined. Studies by one of the authors (Walker) have also indicated that certain perennial species of *Physalis* are susceptible to cucurbit mosaic and the possibility of other sources of infection is indicated by the results of cross inoculations with annual plants of other families. These experiments have shown that cucurbit mosaic may be transmitted to a wide variety of host plants and have furnished evidence which indicates that certain supposedly distinct mosaic diseases may be identical. If this is the case, the possible sources of infection for the cucurbits might perhaps be considerably increased in number. The results of these experiments with annual host plants of the cucurbits and of other families are given in the following section of this paper.

CROSS-INOCULATION EXPERIMENTS WITH CUCURBIT MOSAIC

CROSS INOCULATIONS FROM THE CUCUMBER TO OTHER SPECIES OF THE CUCURBITACEAE

It has been shown in earlier papers (8, 11) that many species of the Cucurbitaceae are susceptible to cucurbit mosaic. In these earlier experiments all of the cucurbits tested proved to be susceptible to the disease, with the exception of those in the genus *Citrullus*. In this genus, which includes the watermelon and citron, infection was produced on the green-seeded citron only. The results with this latter host were also confirmed by the work of Jagger (14). These cross-inoculation experiments have since been continued and a considerable number of additional species and varieties, including many European, Asiatic, and African cucurbits, have been found to be susceptible to the disease. The earlier results have been confirmed in that all varieties except the genus *Citrullus* have proved to be susceptible to the disease. Inoculation of many varieties of watermelon and citron, however, have never resulted in infection, except in the case of the green-seeded citron just mentioned.

The results of these experiments are given in Table XVI, together with a list of the named varieties known to be susceptible to cucurbit mosaic.

TABLE XVI.—*List of cucurbits inoculated with cucurbit mosaic*

Genus and species	Number of varieties inoculated	Number of varieties found susceptible	Source of seed
<i>Cucumis sativus</i> L. (cucumber)	44	44	United States (26), Europe (8), China (4), India (2), Africa (4).
<i>Cucumis anguria</i> L.	1	1	United States.
<i>Cucumis grossulariaeformis</i> Hort.	1	1	United States.
<i>Cucumis metuliferus</i> Mey.	2	2	United States (1), Africa (1).
<i>Cucumis odoratissimus</i> Moench.	1	1	United States.
<i>Cucumis ficifolia</i> Bouche.	2	2	India.
<i>Cucumis melo</i> L. (muskmelon)	17	17	United States (14), Persia, (1), Ceylon (1), Africa (1).
<i>Cucumis melo</i> var. <i>dudaim</i> Naudin.	1	1	United States.
<i>Cucumis melo</i> var. <i>utilissima</i> Roxbg.	1	1	India.
<i>Cucumis melo</i> var. <i>flexuosus</i> Naudin.	1	1	United States.
<i>Cucurbita moschata</i> Duchesne (pumpkin)	2	2	United States.
<i>Cucurbita pepo</i> L. (pumpkin)	10	10	United States (5), Africa (5).
<i>Cucurbita pepo</i> var. <i>condensa</i> Bailey (squash)	6	6	United States (4), India (2).
<i>Cucurbita pepo</i> var. <i>ovifera</i> Bailey (gourd)	5	5	United States.
<i>Cucurbita maxima</i> Duchesne (gourd)	7	7	United States (4), India (3).
<i>Lagenaria vulgaris</i> Ser. (gourd)	5	5	United States.
<i>Lagenaria leucantha</i> Rusby.	1	1	United States.
<i>Luffa cylindrica</i> (aegyptia) Roehm.	6	6	United States (4), India (2).
<i>Luffa acutangulis</i> Roxbg.	2	2	United States (1), India (1).
<i>Benincasa cerifera</i> Cog.	1	1	United States.
<i>Benincasa hispida</i> Cog.	1	1	United States.
<i>Bryonopsis laciniosa</i> Naudin.	1	1	United States.
<i>Echallium elaterium</i> A. Rich.	1	1	United States.
<i>Micrampelis lobata</i> (Michx.) Greene.	1	1	United States.
<i>Momordica charantia</i> L.	5	5	United States (2), India (3).
<i>Momordica involucreta</i> E. Meyer.	1	1	United States.
<i>Sicyos angulatus</i> L.	1	1	United States.
<i>Trichosanthes anguina</i> L.	5	5	United States (2), India (3).
<i>Citrullus vulgaris</i> Schrad. (watermelon and citron) ..	21	21	United States (8), China (2), Africa (11).

The following-named varieties of the more important cultivated cucurbits have been found susceptible to cucurbit mosaic. Only the varieties from the United States and Europe have been listed, as most of the Asiatic and African varieties tested were known only by species names.

CUCUMBER (*Cucumis sativus*): Chicago Pickling, Cumberland, Davis Perfect, Earliest of All, Early Cyclone, Early Fortune, Early Frame, Early Green Cluster, Early Russian, Fordhook Pickling, Giant Pera, Green Prolific, Heinz Pickling, Japanese Climbing, Jersey Pickling, Klondyke, Lemon, Livingston's Emerald, Long Green, Milwaukee Pickling, Parisian Pickling, Small Gherkin, Snow's Pickling, Telegraph, Thorburn's Everbearing, White Spine.

France: Cornichon de Meaux, Cornichon de Toulouse, Vert de Paris.

Holland: Half Long Prolific, King William, Oblong Green Pickling.

Germany: Short Green Early, Short Green Parisian.

CUCUMIS ANGURIA: West Indian gherkin.

MUSKMELON (*Cucumis melo*): Banana Citron, Banquet, Emerald Gem, Hackensack, Hearts of Gold, Honeydew, Long Yellow, Mango, Netted Gem, Orange Christiana, Osage, Rocky Ford, Shumway Giant, Winter Pineapple.

PUMPKIN (*Cucurbita moschata*): Large Cheese, Winter Crookneck.

PUMPKIN (*Cucurbita pepo*): Golden Oblong, Japanese Pie, Mammoth King, Small Sugar, Vegetable Marrow.

SQUASH (*Cucurbita pepo* var. *condensa*): Cocozell Bush, Delicata, Summer Crookneck, White Scallop Bush.

SQUASH (*Cucurbita maxima*): Mammoth Hubbard, Mammoth Whale, Striped Custard.

GOURDS (*Cucurbita pepo* var. *ovifera*): Dipper, Egg, Orange, Pear, Turk's Turban.
GOURDS (*Lagenaria vulgaris*): Bottle, Calabash, Hercules Club, Spoon, Sugar Trough.

GOURDS (*Luffa cylindrica*): Dish Cloth, Sponge.

CITRON (*Citrullus vulgaris*): Green-seeded citron.

The following varieties of watermelon and citron (*Citrullus vulgaris*) have been found to be apparently immune to cucurbit mosaic:

WATERMELON: Halbert Honey, Kleckley Sweet, Mammoth Santiago, Olds 1908, Tom Watson, Sweet Heart.

CITRON: Red-seeded citron.

From 8 to 50 inoculations have been made with each variety, using both insects and artificial methods of inoculation. A considerable number of cross inoculations have also been made between the various species found to be susceptible to the disease. All but one variety of those tested in the genus *Citrullus* seemed to be nonsusceptible to the disease. As far as the writers are aware, no evidence of mosaic infection has ever been obtained on watermelons inoculated either by means of aphids or by artificial methods, although many varieties from many parts of the world have been used in these experiments. Since both Allard (1) and Nishimura (17) have presented evidence which tends to show that certain species of the Solanaceae may carry the infective principle of mosaic without showing visible symptoms, a number of inoculations were made to both cucumber and muskmelon from watermelons which had been inoculated with cucurbit mosaic. These inoculations were made both by artificial methods and by aphids, chiefly the latter, but no evidence was obtained which would indicate that the watermelon may carry the disease without the appearance of visible symptoms.

CROSS INOCULATIONS FROM THE CUCUMBER TO PLANTS OF OTHER FAMILIES

EXPERIMENTS WITH MARTYNIA

The susceptibility of the martynia (*Martynia louisiana*) to cucurbit mosaic has been briefly noted in an earlier paper (8). Although earlier inoculations indicated that the disease could not be transmitted to this host, it was later found that the martynia could be readily infected with mosaic when aphids were used as a means of inoculation (pl. 5, A, B). A continuation of these experiments has given definite evidence of the susceptibility of the martynia to cucurbit mosaic. Further experiments have also shown that the disease may be transmitted from the cucumber to the martynia by artificial inoculations as well as by aphids. The results of these inoculations are given in Table XVII.

The susceptibility of the martynia to cucurbit mosaic under field conditions has been demonstrated by experiments in which martynia plants were grown between rows of cucumbers in the field. The cucumbers developed mosaic early in the season, and a number of the martynias also became infected with the disease after its appearance on the cucumber. Inoculations from these plants to healthy cucumbers resulted in a high percentage of infection. The susceptibility of the cucumber to the mosaic disease found on the martynia has also been proved by inoculations from martynia plants inoculated from mosaic cucumbers as well as from those found naturally infected in the field. As shown in Table XVIII, the disease is transmissible from the martynia to the cucumber by either method of inoculation.



HEALTHY AND MOSAIC MARTYNIA PLANTS

A. Mosaic martynia plant, *Martynia louisiana*, inoculated by means of aphids from a mosaic cucumber plant

B. Healthy martynia plant used as control in above experiment. Madison, Wis., August, 1921

TABLE XVII.—Results of inoculations of healthy plants of *Martynia louisiana* with cucurbit mosaic

Date of inoculation	Method of inoculation	Location of plants	Number of plants inoculated	Number of plants mosaic	Date observed
Sept. 2, 1919	Aphids from mosaic martynia	Greenhouse	9	6	Sept. 12, 1919
Do	Aphids from healthy martynia (control)	do	8	0	Sept. 20, 1919
Apr. 10, 1920	Aphids from mosaic martynia	do	5	3	Apr. 30, 1920
Do	Aphids from healthy martynia (control)	do	5	0	Do.
Apr. 15, 1920	Artificial	do	6	4	Apr. 24, 1920
Do	Control	do	6	0	Do.
Apr. 28, 1920	Aphids from mosaic martynia	do	3	3	May 10, 1920
Do	Aphids from healthy martynia (control)	do	3	0	Do.
July 8, 1920	Artificial	Field	8	5	July 21, 1920
Do	Control	do	8	0	Do.
July 22, 1920	Aphids from mosaic martynia	do	6	2	Aug. 3, 1920
Do	Aphids from healthy martynia (control)	do	6	0	Do.
Aug. 8, 1920	Aphids from mosaic martynia	do	4	4	Aug. 24, 1920
Do	Aphids from healthy martynia (control)	do	4	0	Do.
Aug. 12, 1920	Artificial	Greenhouse	8	6	Do.
Do	Control	do	8	0	Do.

TABLE XVIII.—Results of inoculation of healthy cucumber plants from mosaic plants of *Martynia louisiana*

Date of inoculation	Method of inoculation	Location of plants	Number of cucumber plants inoculated	Number of plants mosaic	Date observed
July 23, 1919	Aphids from mosaic martynia	Greenhouse	12	12	Aug. 4, 1919
Do	Aphids from healthy martynia (control)	do	12	0	Do.
Sept. 3, 1919	Artificial	do	8	6	Sept. 14, 1919
Do	Control	do	8	0	Do.
Do	Artificial	Field	10	8	Do.
Do	Control	do	8	0	Do.
July 22, 1920	Aphids from mosaic martynia	Greenhouse	8	5	July 31, 1920
Do	Aphids from healthy martynia (control)	do	8	0	Do.
July 29, 1920	Artificial	do	6	3	Aug. 7, 1920
Do	Control	do	6	0	Do.
Do	Artificial	Field	10	5	Do.
Do	Control	do	10	0	Do.
Aug. 2, 1920	Artificial	Greenhouse	6	5	Aug. 13, 1920
Do	Control	do	6	0	Do.
Aug. 10, 1920	Aphids from mosaic martynia	do	4	3	Aug. 18, 1920
Do	Aphids from healthy martynia (control)	do	4	0	Do.
Oct. 10, 1920	Artificial	do	6	4	Oct. 27, 1920
Do	Control	do	6	0	Do.
Aug. 10, 1922	Artificial	Field	5	3	Aug. 18, 1922
Do	Control	do	5	0	Do.
Aug. 11, 1922	Artificial	do	6	4	Aug. 22, 1922
Do	Control	do	4	0	Do.

SYMPTOMS OF CUCURBIT MOSAIC ON THE MARTYNIA

The symptoms of the disease on martynia are very similar to those on the cucurbits. Mosaic martynia plants develop a curling and mottling of the younger leaves which is much like that which occurs on the cucumber, with the exception that the green areas are very large and few in number, the yellowed portion including the greater part of the leaf. It has also been noted that a single branch of a mosaic martynia plant will show striking symptoms of mosaic, while

the remainder of the plant shows no evidence of the disease. Inoculations from various parts of such plants to healthy martynias have indicated that the infective principle is localized in the branches, which show visible symptoms of the disease. Eventually, however, the entire plant develops definite symptoms of mosaic. While these cases are somewhat abnormal in martynia, they are of great interest, since in all other plants which have been infected with cucurbit mosaic, the symptoms of the disease manifest themselves at all growing points at approximately the same time. Mosaic martynia plants are much dwarfed when infected with mosaic and the older leaves wilt and die in a manner very similar to those of the cucumber (pl. 5, A, B). In most cases the fruits are much dwarfed but show no mottling, but in one instance plants were found whose fruits were slightly mottled with yellow and showed wartlike protuberances very similar to those found on mosaic cucumber fruits.

EXPERIMENTS WITH PEPPER PLANTS

CROSS INOCULATIONS FROM MOSAIC CUCUMBERS TO THE PEPPER

The appearance of a mosaic disease on pepper plants (*Capsicum annuum* L.) which were growing in the vicinity of mosaic cucurbits led to the first cross inoculations with this host. The cucumber aphid will occasionally feed on the pepper, and it was found that cucurbit mosaic could be transmitted to the pepper by means of these insects. As in the case of martynia, the disease was later successfully transmitted to the pepper by artificial methods of inoculation, but the percentage of infection has always been considerably lower by this method than when aphids were used. The results of the inoculations from the cucumber to the pepper are given in Table XIX. As in the case of martynia, there is considerable field evidence of the transmission of mosaic from the cucumber to the pepper. Where pepper plants are grown next to mosaic cucumber plants in the field, a large percentage of the plants have always shown symptoms of mosaic before the end of the season. The experimental plot in which these experiments were conducted was at a considerable distance from any fields of tobacco or tomatoes and there was no reason to believe that the infection on the pepper had come from any source other than the cucumber.

CROSS INOCULATIONS FROM MOSAIC PEPPERS TO THE CUCUMBER

Cross inoculations from mosaic peppers to healthy cucumber plants have been successful, as shown by the data in Table XX. These inoculations were made from peppers inoculated from mosaic cucumber plants and also from those found naturally infected with mosaic in the field. The inoculations from naturally infected pepper plants included those grown near mosaic cucurbits and presumably infected from that source and also mosaic plants found at other points where there were no mosaic cucurbits in the neighborhood. Infection was brought about both by artificial methods of inoculation and by the use of aphids, but the latter method was considerably more successful although artificial inoculations have given a considerably higher percentage of infection than in the case of the inoculations from cucumber to pepper.

TABLE XIX.—Results of cross inoculation of healthy pepper plants with cucurbit mosaic

Date of inoculation	Method of inoculation	Location of plants	Number of plants inoculated	Number of plants mosaic	Date observed
May 8, 1920	Aphids from mosaic cucumber plant	Greenhouse	6	4	May 15, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
May 18, 1920	Aphids from mosaic cucumber plant	do	6	5	May 27, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
May 28, 1920	Aphids from mosaic cucumber plant	do	8	8	June 10, 1920
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.
July 19, 1920	Aphids from mosaic cucumber plant	do	4	4	Aug. 2, 1920
Do	Aphids from healthy cucumber plant (control).	do	4	0	Do.
July 20, 1920	Aphids from mosaic cucumber plant	do	7	7	July 28, 1920
Do	Aphids from healthy cucumber plant (control).	do	7	0	Do.
July 23, 1920	Artificial	do	4	3	July 31, 1920
Do	Control	do	4	0	Do.
July 24, 1920	Artificial	do	6	2	Aug. 2, 1920
Do	Control	do	6	0	Do.
July 29, 1920	Artificial	Field	19	8	Aug. 10, 1920
Do	Control	do	9	0	Do.
Do	Artificial	Greenhouse	6	2	Do.
Do	Control	do	6	0	Do.
Aug. 2, 1920	Aphids from mosaic cucumber plant	do	6	6	Aug. 10, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
Aug. 4, 1920	Aphids from mosaic cucumber plant	do	6	6	Aug. 11, 1920
Do	Aphids from healthy cucumber plant (control).	do	6	0	Do.
Aug. 18, 1920	Artificial	do	8	3	Sept. 1, 1920
Do	Control	do	8	0	Do.
Do	Aphids from mosaic cucumber plant	do	8	6	Do.
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.
Sept. 2, 1920	Artificial	Field	18	8	Sept. 23, 1920
Do	Control	do	18	0	Do.
Aug. 10, 1922	Artificial	do	12	5	Aug. 22, 1922
Do	Control	do	8	0	Do.
Aug. 14, 1922	Aphids from mosaic cucumber plant	do	8	8	Aug. 24, 1922
Do	Aphids from healthy cucumber plant (control).	do	8	0	Do.

TABLE XX.—Results of cross inoculations from mosaic pepper to healthy cucumber plants

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Apr. 28, 1920	Aphids from mosaic pepper plant	Greenhouse	18	18	May 15, 1920
Do	Aphids from healthy pepper plant (control).	do	14	0	Do.
July 8, 1920	Aphids from mosaic pepper plant	Field	3	3	July 20, 1920
Do	Aphids from healthy pepper plant (control).	do	3	0	Do.
July 19, 1920	Aphids from mosaic pepper plant *	do	6	6	Aug. 1, 1920
Do	Aphids from healthy pepper plant (control).	do	6	0	Do.
Mar. 26, 1922	Aphids from mosaic pepper plant	Greenhouse	18	13	Apr. 7, 1922
Do	Aphids from healthy pepper plant (control).	do	10	0	Do.
May 1, 1922	Aphids from mosaic pepper plant	do	28	21	May 10, 1922
Do	Aphids from healthy pepper plant (control).	do	14	0	Do.
May 11, 1922	Artificial	do	8	4	May 25, 1922
Do	Control	do	8	0	Do.
June 10, 1922	Artificial	do	10	5	June 21, 1922
Do	Control	do	10	0	Do.
Aug. 8, 1922	Aphids from mosaic pepper plant *	do	4	4	Aug. 21, 1922
Do	Aphids from healthy pepper plant (control).	do	4	0	Do.
Apr. 15, 1923	Artificial	do	4	2	Apr. 25, 1923
Do	Control	do	4	0	Do.

* Pepper plants found naturally infected in the field.

SYMPTOMS OF CUCURBIT MOSAIC ON THE PEPPER

When inoculated with cucurbit mosaic, the symptoms of the disease develop in the pepper in a manner which is very similar to that noted in the cucumber. The younger leaves of the infected plant curl downward along the midrib and the basal portion of the leaf is frequently a lighter green than that at the tip. Within a short time, however, such leaves develop the mottled appearance characteristic of the disease. The contrast in color, however, is seldom as pronounced as in the cucurbits and the mottling of the leaves is not general as in the cucumber, as only the very young leaves and those produced after infection are affected in this way. When pepper plants have been mosaic diseased for some time, the leaves are considerably smaller than those of healthy plants of the same age. The stem internodes are shortened considerably and the length of the petioles is somewhat reduced. Mosaic plants have a more compact habit of growth than normal plants, and the leaves are often abnormally narrow and drawn out in filiform fashion at the tip (pl. 6, A). This character of growth often produces an almost rosettelike character in plants which are infected when young. The foliage of mosaic peppers becomes a yellowish green, and the leaves appear to be firmer in texture than those of healthy plants. The mottling of the leaves is also less pronounced in plants which have been infected for some time and may be so slight as to be almost indistinguishable except upon close examination. In a few cases the fruits of mosaic pepper plants have shown symptoms which are comparable to those found on the fruits of the cucumber (pl. 3, B, C, D). The greater part of such fruits retain their normal green color, but the surface is broken with dark-green areas which are raised above the surface in warty swellings similar to those found on cucumbers affected with mosaic.

INTERTRANSMISSION OF CUCUMBER AND TOBACCO MOSAIC

The discovery of the susceptibility of the pepper to cucurbit mosaic has been of particular interest, since both Allard (1) and Schwarze (18) have shown that the pepper is also susceptible to the mosaic disease of tobacco. A comparative study was made, therefore, between pepper plants inoculated with tobacco mosaic and those inoculated from mosaic cucumber plants. These experiments indicated that pepper plants inoculated from mosaic plants of either of these hosts behaved in essentially the same manner. The period of incubation was the same and the symptoms appeared to be of the same type in both cases. The plants were dwarfed, and the leaf symptoms were the same as those described above (pl. 6, A, B, C). The pepper is also susceptible to tomato mosaic and it was found that peppers inoculated from mosaic tomato plants also showed symptoms apparently the same as those found on peppers inoculated from mosaic cucumber plants. This would be expected; in view of the results with tobacco, the infective agency of tobacco and tomato mosaic being apparently the same.

Since the pepper was susceptible to both cucumber and tobacco mosaic, the possibility was suggested that the mosaic of one of these hosts might be transmitted to plants of the other host through the



HEALTHY AND MOSAIC PEPPER PLANTS

A. Mosaic pepper plant inoculated with cucumber mosaic, showing distortion of foliage and slightly dwarfed character of growth

B. Mosaic pepper plant inoculated with tobacco mosaic on same date as plant in A, and showing similar symptoms

C. Healthy pepper plant used as control in above experiment. Madison, Wis., August, 1923

pepper. Such a possibility was of interest, owing to the fact that a large number of inoculations had been made from mosaic cucumber plants to tobacco, and vice versa, without obtaining any evidence that either of these mosaic diseases was transmissible to the other host. The symptoms on the pepper were so similar, however, when the plants were inoculated either with tobacco or cucumber mosaic, that it seemed possible that the diseases on tobacco and cucumber were at least intertransmissible if not identical.

Experiments were undertaken, therefore, in which pepper plants were inoculated with cucurbit mosaic, both by means of aphids and by artificial methods of inoculation. The plants used in these experiments were grown in a greenhouse which contained no other mosaic plants and were kept covered with cheesecloth cages during the experiment. Control plants were kept under separate cages in each series of inoculations. After mosaic symptoms had developed on the peppers thus inoculated with cucurbit mosaic, inoculations were made from them to healthy cucumber and tobacco plants. The same pepper plant was used as a source of inoculum for both hosts and control plants were placed under separate cages. In the case of the cucumber, the inoculations were made by means of aphids and also by artificial methods, using the juices of the mosaic pepper. The tobacco plants were inoculated only by the latter method. In all of these experiments, as shown in Table XXI, a high percentage of infection occurred on both cucumber and tobacco plants when inoculated from peppers affected with cucurbit mosaic. The controls remained healthy in all cases. The symptoms produced on the tobacco plants appeared to be the same as those produced on other tobacco plants inoculated with tobacco mosaic for comparison, and the incubation period was the same in both cases. This also held true in the case of the incubation period of cucumbers inoculated from mosaic cucumber plants when compared with those inoculated from the pepper.

TABLE XXI.—*Results of cross inoculations of cucumber and tobacco plants from pepper plants infected with cucumber mosaic*

Date of inoculation, 1922	Pepper plant used as inoculum	Plant inoculated	Method of inoculation	Number of plants inoculated	Number of plants mosaic	Date observed, 1922
Aug. 19	2-e (mosaic)	Tobacco	Artificial	10	6	Aug. 27
Do.	1 (healthy)	do	Control	10	0	Do.
Do.	2-e (mosaic)	Cucumber	Artificial	6	4	Aug. 26
Do.	do	do	Aphids from mosaic pepper plant.	6	6	Do.
Do.	1 (healthy)	do	Aphids from healthy pepper plant (control).	6	0	Do.
Oct. 4	3-H (mosaic)	Tobacco	Artificial	10	7	Oct. 12
Do.	4 (healthy)	do	Control	10	0	Do.
Do.	3-H (mosaic)	Cucumber	Artificial	9	4	Oct. 10
Do.	do	do	Aphids from mosaic pepper plant.	9	9	Do.
Do.	4 (healthy)	do	Aphids from healthy pepper plant (control).	9	0	Do.
Nov. 7	6-R (mosaic)	Tobacco	Artificial	6	5	Nov. 15
Do.	2 (healthy)	do	Control	6	0	Do.
Do.	6-R (mosaic)	Cucumber	Artificial	8	4	Do.
Do.	2 (healthy)	do	Control	6	0	Do.

Inasmuch as these experiments indicated that cucumber mosaic could be transmitted to tobacco through the use of the pepper as an intermediate host, another series of inoculations was made in which

peppers were inoculated with the expressed juice of mosaic tobacco plants and the inoculations later made from these plants to both cucumber and tobacco. All of these inoculations were made by the artificial method in the case of tobacco, but the cucumber plants in some cases were also inoculated by means of aphids as in the preceding experiments. The results of these experiments (Table XXII) indicate that cucurbit mosaic can be transmitted to tobacco through the pepper, and that tobacco mosaic can be transmitted in similar manner to the cucumber.⁵ These results seem to give some support to the belief that the causal agencies of these diseases are identical, but as yet this hypothesis can not be considered as proved.

TABLE XXII.—Results of cross inoculation of cucumber and tobacco plants from pepper plants infected with tobacco mosaic

Date of inoculation	Pepper plant used as inoculum	Plant inoculated	Method of inoculation	Number of plants inoculated	Number of plants mosaic	Date observed
Oct. 17, 1922	3-a (mosaic)	Tobacco	Artificial	8	6	Oct. 26, 1922
Do	10 (healthy)	do	Control	8	0	Do.
Do	3-a (mosaic)	Cucumber	Aphids from mosaic pepper plant.	8	5	Do.
Do	10 (healthy)	do	Aphids from healthy pepper plant (control).	8	0	Do.
Nov. 14, 1922	2-c (mosaic)	Tobacco	Artificial	8	5	Nov. 22, 1922
Do	12 (healthy)	do	Control	8	0	Do.
Do	2-c (mosaic)	Cucumber	Artificial	8	5	Nov. 20, 1922
Do	do	do	Aphids from mosaic pepper plant.	8	8	Do.
Do	12 (healthy)	do	Aphids from healthy pepper plant (control).	8	0	Do.
Mar. 23, 1923	4-d (mosaic)	Tobacco	Artificial	8	6	Mar. 31, 1923
Do	7 (healthy)	do	Control	8	0	Do.
Do	4-d (mosaic)	Cucumber	Artificial	10	6	Mar. 30, 1923
Do	do	do	Aphids from mosaic pepper plant.	10	8	Do.
Do	7 (healthy)	do	Aphids from healthy pepper plant (control).	10	0	Do.

CROSS-INOCULATION EXPERIMENTS WITH THE PIGWEED

CROSS INOCULATION FROM MOSAIC PIGWEED TO THE CUCUMBER

Field observations at Madison during the summer of 1920 showed that pigweeds (*Amaranthus retroflexus*) which were growing near mosaic cucumbers were affected with a mosaiclike disease. The affected plants were dwarfed, the leaves were irregular in shape, and the younger leaves were mottled in a manner typical of the mosaic diseases. An examination also showed that these plants were infested with aphids, which were also abundant on the nearby mosaic cucumber plants. Aphids from the supposedly mosaic pigweed were transferred to healthy cucumber plants under cages in the greenhouse, and mosaic symptoms later developed on the majority of these plants. Control plants with aphids from healthy pigweeds, however, remained free from mosaic. In order to eliminate the possibility that some of the aphids on the pigweed had recently come from mosaic cucumber plants and were carrying

⁵ Since these experiments were undertaken. O. H. Elmer, of the Iowa Agricultural Experiment Station, has reported that he has secured infection in tobacco plants directly inoculated with cucurbit mosaic. (13)

the disease from the cucumber rather than the pigweed on which they were collected, further experiments were undertaken in which mosaic pigweeds were first freed from aphids by spraying and then covered with insect-proof cages. After sufficient time had elapsed to insure the plants being free from insects, aphids were transferred to the pigweed from healthy cucumber plants. At the same time, other aphids from the same healthy cucumber plants were placed on healthy pigweeds under separate cages. Ten days later the aphids on both the healthy and mosaic pigweeds were transferred to separate lots of healthy cucumber plants under cages in the greenhouse. The cucumbers on which the aphids from the mosaic pigweeds were placed developed mosaic in a majority of cases, while the control plants remained healthy. Inoculations were also made by artificial methods from mosaic pigweeds to cucumber plants, both in the field and greenhouse. In these inoculations the crushed leaf tissues of the mosaic plant, inserted in longitudinal incisions made in the stem of the cucumber, were used as inoculum. As shown in Table XXIII, a high percentage of infection resulted from this method as well as from the use of aphids.

TABLE XXIII.—*Cross inoculations from mosaic plants of Amaranthus retroflexus to healthy cucumbers*

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Aug. 12, 1920	Aphids from mosaic pigweed plant	Greenhouse	6	6	Aug. 22, 1920
Do	Aphids from healthy pigweed plant (control).	do	6	0	Do.
Aug. 13, 1920	Artificial	Field	8	6	Aug. 25, 1920
Do	Control	do	8	0	Do.
Aug. 18, 1920	Aphids from mosaic pigweed plant	do	8	7	Sept. 1, 1920
Do	Aphids from healthy pigweed plant (control).	do	6	0	Do.
Do	Aphids from mosaic pigweed plant	Greenhouse	7	7	Do.
Do	Aphids from healthy pigweed plant (control).	do	7	0	Do.
Do	Artificial	do	8	2	Do.
Do	Control	do	6	0	Do.
Do	Artificial	Field	12	3	Do.
Do	Control	do	8	0	Do.
Sept. 2, 1920	Artificial	do	10	6	Sept. 13, 1920
Do	Control	do	6	0	Do.
Sept. 4, 1920	Artificial	do	6	5	Do.
Do	Control	do	6	0	Do.
Do	Artificial	Greenhouse	4	1	Do.
Do	Control	do	4	0	Do.
July 15, 1922	Aphids from mosaic pigweed plant	do	12	12	July 25, 1922
Do	Aphids from healthy pigweed plant (control).	do	10	0	Do.
June 30, 1923	Artificial	do	6	3	July 7, 1923
Do	Control	do	6	0	Do.

CROSS INOCULATION FROM MOSAIC CUCUMBER PLANTS TO THE PIGWEED

As a result of the successful inoculations from mosaic *Amaranthus* plants to the cucumber, efforts were made to transmit cucurbit mosaic to this host. Aphids from mosaic cucumber plants were transferred to the leaves of healthy pigweeds growing under cages in the greenhouse, and aphids from healthy cucumber plants were placed on other pigweeds as controls. The inoculated plants had been grown from seed in the greenhouse and were from 6 to 12 inches tall. At the end of 7 days the inoculated plants showed a slight

downward curling of the youngest leaves, and at the end of 12 days a definite mottling began to appear. In the course of the next 2 weeks the mosaic plants developed typical mosaic symptoms and the leaves were mottled and deformed as the new growth developed. The early symptoms of the disease on the pigweed are less marked than with any plants on which the writers have obtained infection, and for this reason the incubation period at first appears to be longer than it really is. As the experiments have been continued it has become evident that the incubation period for this host is approximately the same as for the cucurbits, varying from 7 to 9 days.

As shown in Table XXIV, infection has been accomplished on the pigweed by both methods of inoculation. The artificial inoculations were made in the same manner as those used in inoculating cucumber plants from mosaic pigweeds and a fair amount of infection resulted from this method. The results of these experiments indicate that the pigweed is readily infected with cucurbit mosaic and that such infection occurs under natural conditions in the field. The occurrence of such natural infection has often been observed in fields where pigweeds were growing between rows of cucumbers affected with mosaic. Up to the present the only mosaic pigweeds which have been found have apparently been infected from the cucumber and there is no evidence to indicate that the disease is carried over winter by this host.

TABLE XXIV.—Cross inoculations from mosaic cucumbers to healthy plants of *Amaranthus retroflexus*

Date of inoculation	Method of inoculation	Location	Number of plants inoculated	Number of plants mosaic	Date observed
Apr. 21, 1921	Aphids from mosaic cucumber plant . . .	Greenhouse .	1	1	May 9, 1921
July 14, 1922	do . . .	do . . .	4	4	July 31, 1922
Do . . .	Aphids from healthy cucumber plant (control) .	do . . .	4	0	Do.
July 19, 1922	Aphids from mosaic cucumber plant . . .	do . . .	8	8	Aug. 1, 1922
Do . . .	Aphids from healthy cucumber plant (control) .	do . . .	8	0	Do.
Aug. 2, 1922	Artificial . . .	do . . .	10	6	Aug. 18, 1922
Do . . .	Control . . .	do . . .	10	0	Do.
Aug. 10, 1922	Artificial . . .	do . . .	5	3	Aug. 28, 1922
Do . . .	Control . . .	do . . .	5	0	Do.

SYMPTOMS OF CUCURBIT MOSAIC ON AMARANTHUS RETROFLEXUS

Mosaic *Amaranthus* plants are much dwarfed and usually reach a height of only 8 to 10 inches, while healthy plants are often 2 to 3 feet tall. The affected plants show a typical mosaic mottling of the foliage, the major portion of the leaf becoming a yellowish-green with smaller dark-green areas scattered over the surface. The petioles of such leaves are usually longer in proportion to the blade than those of healthy plants, and this, together with the shortening of the internodes, gives the mosaic plant an abnormally spreading habit (pl. 3, E). The leaves of mosaic plants are usually smaller, narrower, and more irregular in outline than those of healthy plants. Mosaic plants usually develop only a single stem and do not branch as commonly as do healthy pigweeds. Seed is produced by mosaic plants, but only in small amounts.

Observations have shown that another species of pigweed, *Amaranthus blitoides* Wats., occasionally shows symptoms which have somewhat the appearance of a mosaic disease, but up to the present the writers have not been able to demonstrate that it is transmissible.

CROSS-INOCULATION EXPERIMENTS WITH PLANTS OF OTHER GENERA

In the course of the studies on the overwintering of cucurbit mosaic, a number of plants of various families have been found to show symptoms of what appear to be mosaic diseases. Symptoms of this type have been noted on a perennial species of *Helianthus* in a number of cases, but no infection has been produced on the cucumber from this host, nor has the disease been transmitted to healthy plants of the same species. Symptoms similar to those of mosaic have also been found on *Arctium lappa* L., *Abutilon theophrasti* Medic., *Malva rotundifolia* L., *Oxybaphus nyctagineus* (Michx.) Sweet, *Ambrosia trifida* L., *Bidens vulgata* Greene, and on species of *Anemone*, *Xanthium*, and wild *Rubus*. A limited number of inoculations have been made with all of these plants, but up to the present the writers have no evidence which indicates that these are transmissible mosaic diseases. These experiments are being continued, however, and it may be found that certain of these hosts are affected with mosaic.

RELATION OF ANNUAL HOST PLANTS TO THE OVERWINTERING AND DISSEMINATION OF CUCURBIT MOSAIC

The annual host plants of cucurbit mosaic would not appear to be concerned in the overwintering of the disease unless the seed of such plants carries the disease from year to year. Experiments have been conducted with seed from annual plants outside the Cucurbitaceae which are known to be susceptible to cucurbit mosaic—pepper, martynia, pigweed, and cultivated *Physalis*. From 300 to 700 seedlings have been grown from seed of mosaic plants of each of the above hosts, but there has been no evidence that the disease is transmitted through the seed. For this reason, it is felt that the perennial wild hosts are probably the only ones concerned in the overwintering of the disease, with the exception of the wild cucumber.

The annual host plants of cucurbit mosaic may be of importance, however, as sources of infection to the cultivated cucurbits during the growing season. In the course of experiments on the control of cucurbit mosaic through the eradication of the perennial wild host plants, it has been found that the disease may be contracted by annuals from the perennials and carried on the annual plants during the summer after the perennial wild hosts have been destroyed. Field observations have shown this to be true in the case of *Amaranthus retroflexus* and cultivated species of *Physalis*, and these host plants have apparently acted as sources of infection to adjacent cucurbits throughout the season. It is evident, therefore, that these annual hosts are of practical importance from the standpoint of the control of the disease.

RELATION OF WILD HOST PLANTS TO THE CONTROL OF CUCURBIT MOSAIC

As cucurbit mosaic is so readily transmitted by insects in the field, it has proved difficult to check it after it once appears in the fields to any appreciable extent. For this reason, the most prac-

ticable method of controlling the disease has appeared to consist of the early removal of the wild host plants which represent the original sources of infection. Field experiments have been in progress for the past three years in an effort to determine the practical possibilities of controlling the disease by the eradication of these wild hosts and the work is being continued. As a result of this work the host range of cucurbit mosaic has been found to be larger than was at first suspected and the eradication program has consequently been somewhat enlarged. The evidence obtained up to the present indicates definitely that wild host plants are probably responsible for practically all the primary infection on the cultivated cucurbits. The practical possibilities of controlling the disease by the eradication of these host plants have not as yet been fully worked out, but recent results have offered considerable encouragement.

SUMMARY

Continued investigations of the overwintering of cucurbit mosaic have shown that the disease does not persist in the soil.

Further trials with the seed of mosaic cucumber, squash, muskmelon, and pumpkin plants indicate that seed transmission of the disease probably does not occur or is so rare as to be of little significance.

There is no evidence that the striped beetle (*Diabrotica vittata*) is an agency in overwintering cucurbit mosaic.

Cucurbit mosaic is transmissible through the seed of the wild cucumber (*Micrampelis lobata*) and the disease is transmitted from this host to the cultivated cucurbits by the cucumber aphid (*Aphis gossypii*), the striped beetle, (*Diabrotica vittata*), and the 12-spotted beetle (*D. 12-punctata*). The striped beetle is the chief agency in transmitting the primary infection from the wild cucumber to the cultivated cucurbits, as this insect first feeds on the wild host and later migrates to the cultivated cucumbers. Field experiments indicate that the beetles may thus carry infection over distances of at least 400 yards.

Surveys of cucumber-growing districts in Wisconsin and northern Illinois indicate that the wild cucumber is an important factor in overwintering mosaic.

The milkweed (*Asclepias syriaca*) is susceptible to cucurbit mosaic, and is important as a means of overwintering the disease. Mosaic lives over in the roots of the milkweed, and mosaic plants are often found in the vicinity of cucumber fields. The cucumber aphid feeds on the milkweed and acts as a carrier of the disease from this host to the cucumber. Observations indicate that most mosaic milkweeds are originally infected from adjacent cucumbers. In many localities the milkweed appears to be of more importance than the wild cucumber as a source of infection to the cultivated cucurbits, as the mosaic plants usually occur in the immediate vicinity of the fields.

The pokeweed (*Phytolacca decandra*) is susceptible to cucurbit mosaic, and the disease found on pokeweeds in the field is transmissible to the cucumber. Mosaic lives over winter in the roots of the pokeweed, and the cucumber aphid acts as a carrier of the disease from the pokeweed to the cucurbits. Mosaic pokeweeds are rare in

Wisconsin but occur in southern Michigan and northern Indiana. The pokeweed is common in southern Indiana and in the cucumber-growing sections of southern Illinois, where it seems to be an important source of infection to the cucurbits.

Cucurbit mosaic also lives over winter in the roots of catnip (*Nepeta cataria*) and certain perennial species of *Physalis*.

In cross-inoculation experiments with plants of the Cucurbitaceae, all species tested have proved to be susceptible to mosaic except those of the genus *Citrullus*. Infection has been produced in 11 genera, including 23 species and 96 horticultural varieties.

Successful cross inoculations have been made from mosaic cucumbers to the *Martynia louisiana*, pigweed (*Amaranthus retroflexus*), and pepper (*Capsicum annuum*).

The pepper is also susceptible to tobacco mosaic, and it has been found that pepper plants may be infected with tobacco mosaic and the disease then transmitted from them to either tobacco or cucumber. Similar results were obtained from pepper plants inoculated with cucurbit mosaic, and the results indicate that the infective principle of tobacco and cucurbit mosaic are intertransmissible.

There is no evidence of seed transmission of mosaic in the case of martynia, pepper, or pigweed, and as these plants are annuals they do not seem to be concerned in overwintering mosaic. These annual hosts, when infected with mosaic, however, often act as sources of infection to cucurbits during the summer.

Experiments on the control of cucurbit mosaic by removal of the wild hosts on which it overwinters are now in progress.

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PRELIMINARY REPORT ON COLLOIDAL CLAYS AS EMULSIFIERS FOR MINERAL OILS USED IN SPRAYING CITRUS GROVES ¹

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INTRODUCTION

Soap emulsions of mineral lubricating oils have been used for many years in Florida for spraying citrus trees for the control of several species of scale insects and white flies. These are usually made according to the following formula:

Mineral oil.....	gallons..	2
Water.....	do.....	1
Caustic potash fish oil soap.....	pounds..	2

Several mineral oils have been used, but those used most extensively have the following physical specifications:

Oil	Specific gravity at 27° C.	Flash point (° C.)	Fire point (° C.)	Viscosity ¹	Volatility ²
Medium.....	0.886	184	207	365.3	4.9
Heavy.....	.896	163	245	1,121.0	.16

¹ Engler, H₂O=100.

² 1 gram for 4 hours at 105° C.

The materials are put into a kettle or other vessel that will stand fire and are heated to the boiling point—about 168° F. After removal from the fire, but while still very hot, the mixture is pumped with a bucket pump from the kettle to another receptacle, and back again. The formula just mentioned is sufficient for 197 gallons of water, making about 1 per cent of oil in the diluted spray material.

These soap-mineral oil emulsions have given most satisfactory results from the standpoint of insect control. The simple soap emulsions, however, have certain limitations which make them somewhat inconvenient for citrus growers. For example, they are not sufficiently stable to mix with "hard" or deep-well water unless this water is first treated with chemicals to "soften" it; neither will they mix with lime-sulphur solution without the emulsions themselves being specially treated with a stabilizing agent. They will, however, mix with alkaline Bordeaux mixture in all proportions.

The simple soap emulsions can be made to mix with any "hard" or deep-well water found in Florida citrus groves or with lime-sulphur solution by means of stabilizers such as glue, casein, milk powder, flour, corn starch, laundry starch, and water glass. When these are placed in the emulsions immediately before they are to be used satisfactory results have always followed; but if the organic materials are put into the emulsions any considerable length of time before they are to be used fermentation usually takes place and the entire emul-

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sion breaks down, resulting in total loss, because it can not be re-emulsified. Except for these defects the soap emulsions have given great satisfaction.

In the search for a nonfermentable stabilizer for soap emulsion experiments were made with a number of inorganic materials, including kaolin, a china clay composed mostly of hydrous aluminum silicate, which did not prove satisfactory for this purpose. Following suggestions of the junior author, these experiments led to the use of it as an emulsifier instead of soap.

KAOLIN AS AN EMULSIFIER

The first attempt by the writers to use the kaolin as an emulsifier resulted in obtaining a perfect emulsion, and thereafter practically all the experimental work was directed to determining the quantity of kaolin required for best results. Oils having the same physical specifications as those previously given were used in all the kaolin emulsion experiments.

The following formulae were tried in preparing the first few lots of kaolin emulsion:

	Mineral lubricating oil	Water	Kaolin
	Gallons	Gallon	Pounds
A	2	1	4
B	2	1	2½
C	2	1	2

PROCEDURE.—Milk of kaolin was made by simply adding the water to the kaolin and permitting the mixture to stand 15 to 30 minutes. The oil was then added to this mixture and emulsified without the aid of heat by passing the materials twice through a bucket pump.

All of the above formulae produced reasonably satisfactory emulsions. The first test, where 4 pounds of kaolin were used, produced a perfect emulsion, but the resulting paste was entirely too stiff for practical grove spraying. Where only 2 pounds were used, the emulsion tested satisfactorily when diluted with water, but there appeared to be some free oil in the emulsion itself, and it was thought advisable to use a little more kaolin. The formula whereby 2½ pounds were used, or 1½ pounds per gallon of oil, proved to be perhaps the most satisfactory, and it was used in practically all extensive spraying work.

Attempts were made to produce an emulsion by only stirring, in much the same manner as in preparing the cold-stirred soap emulsions. The oil was added very slowly and gradually to the milk of kaolin, but the writers were not able to stir it violently enough to produce a satisfactory emulsion. No doubt an emulsion can be made by this method if sufficient stirring is given the mixture and the oil is added very gradually.

FULLER'S EARTH AS AN EMULSIFIER

Fuller's earth, a crude material of variable composition, but mostly of hydrous aluminum silicate, used extensively in the textile and oil industries, was tested for its value as an emulsifying agent for mineral oils. Experiments were conducted to determine whether it is a satisfactory material for emulsifying mineral oil, the following formula being used:

Mineral lubricating oil.....	gallon..	1
Water.....	do.....	1
Fuller's earth.....	pounds..	2 $\frac{2}{3}$

As was the case with kaolin, fuller's earth dispersed readily in the water, after which the oil was added. The entire mixture was pumped three times, and the resulting emulsion proved satisfactory, but it was much thinner than where a similar proportion of kaolin was used. In order to test fuller's earth further, the following formula was tried:

Mineral lubricating oil.....	gallons..	2
Water.....	do.....	1
Fuller's earth.....	pounds..	2 $\frac{2}{3}$

The entire mixture was pumped twice, resulting in a very thick emulsion, which when tested with water gave a little free oil, but further trials gave entirely satisfactory results.

In laboratory tests kaolin and fuller's-earth emulsions were mixed with practically all spray materials used in citrus groves, including 1-1-50, 3-3-50, 5-5-50, neutral and improperly prepared Bordeaux mixtures as well as "hard" deep-well water, and many other solutions. Concentrated sulphuric, nitric, and hydrochloric acids, saturated caustic soda and concentrated lime-sulphur solutions had no appreciable effect on causing the oil to separate.

These emulsions do not separate into layers within a reasonable length of time as do the soap emulsions and commercial miscible oils, nor do they deteriorate rapidly. Kaolin emulsion has been kept in an open barrel for more than three months during the hottest period of the year and it still remained satisfactory for use. Certain changes, however, take place. With age the emulsion changes from a creamy yellow to a bluish color beneath the surface and an offensive sulphurous odor is given off. One lot separated and spoiled after remaining in an open barrel a year, and efforts to reemulsify it by adding water and more kaolin were not successful.

BRICK CLAY AS AN EMULSIFIER

Brick clay was also tested and found to make a perfect emulsion, but because of the presence of more or less sand in the clay it probably would not be a satisfactory emulsifier for spraying emulsions.

In addition to the above-mentioned colloidal clays, hydrated lime and commercial Bordeaux pastes and powders were used, tested in the laboratory only, and found to be reasonably satisfactory but not nearly as good as kaolin or fuller's earth.

KAOLIN LIME-SULPHUR OIL EMULSION

After several unsuccessful attempts, kaolin also proved to be a satisfactory emulsifier to convert lime-sulphur solution and oil into an emulsion. The failures were due to the allowance of insufficient time for the kaolin to become completely dispersed by the lime-sulphur solution. It was thought advisable to emulsify the regular quantities of materials usually recommended for 150 gallons of standard dilution. They are as follows:

Mineral lubricating oil.....	gallons..	1
Lime-sulphur solution.....	do.....	3
Kaolin.....	pounds..	2

When put in the lime-sulphur solution the kaolin did not disperse as quickly as in water, but on standing overnight it went into suspension readily. The oil was added and emulsified with a bucket pump, a satisfactory product resulting.

Fuller's earth was used instead of kaolin to make the following excellent emulsion:

To 3 quarts of lime-sulphur solution 1 pound of fuller's earth was added. The clay formed a suspension very readily in the lime-sulphur solution, after which 3 quarts of oil was added and the materials emulsified by pumping twice.

FIELD EXPERIMENTS

On February 9, 1923, some of the kaolin emulsion was used for experimental spraying on one lemon tree in full bloom and with much new growth, two orange trees, and two wild cherry trees (*Prunus serotina*) in full flush of growth. Six days later the sprayed trees were examined. The cherry trees were quite badly defoliated, but the only damage observed on the citrus trees was the slight curling of six leaves on the lemon. No excessive defoliation had taken place.

Normally, a few of the oldest leaves fall after applications of the oil sprays in general use. Leaf droppage induced by oil sprays seldom if ever occurs until the third day after the spray is applied. The distinctive features of this type of defoliation are as follows: The leaf retains its normal green color, and a few days after the spray is applied the blade, while still green, disjoints and falls from the petiole, leaving it attached to the branch. If the dosage is excessively strong, or if two or more applications of normal dilution are made within two or three weeks without rains to remove the excess oil, or if the emulsion breaks, severe defoliation and fruit droppage is likely to occur.

On February 27, 1923, the same trees that were sprayed 18 days previously were sprayed again, using the kaolin emulsion 1 to 50, or approximately $1\frac{1}{2}$ per cent of oil in the diluted spray. For this work $1\frac{1}{3}$ pounds of kaolin per gallon of oil were used instead of the 1 pound, as of February 9. The material mixed well, but the trees seemed to have a greasy appearance after being sprayed, and the drops of spray were quite large. There were blooms on both the lemon and orange trees on the date of spraying. It rained about 5 hours after the spray had been applied, and also on the following day. Little or no damage was done to these trees.

On February 13, 1923, the following sprays were applied on bearing orange trees:

- 3 quarts simple soap emulsion in 50 gallons of water.
- 3 quarts of kaolin emulsion in 50 gallons of water.
- 3 quarts of kaolin emulsion in 50 gallons of 3-3-50 Bordeaux mixture.
- 3 quarts of kaolin emulsion and 2 pounds of soda-sulphur in 50 gallons of water.
- 3 quarts of kaolin emulsion and 5 quarts of lime-sulphur solution in 50 gallons of water.

At this time ripe fruit was on the trees, a few tiny bloom buds (not full white) were present, and there was considerable new growth. Two days later all of the trees were carefully examined to determine if any damage had been done. No damage whatever was observed. The fruit, new leaves, and tiny buds remained normal, and there was no excessive shedding of old leaves.

Again, on May 11, 1923, 13 seedling orange and grapefruit trees near Orlando, Fla., were sprayed with kaolin emulsion at the rate of 9 quarts to 150 gallons of water, or approximately 1 per cent oil in the diluted spray. The grapefruits were nearly $1\frac{1}{2}$ inches in diameter, and the oranges about $1\frac{1}{8}$ inches. The sun was bright and the temperature approximately 80° F. The emulsion mixed perfectly with the lake water and there was no residue in the tank when the load was finished. The spray collected in large drops on the fruit and foliage and they appeared to be covered with free oil. A few minutes after spraying spots (commonly called "shadows") with an oil-soaked appearance developed where the drops of spray had collected. Some of the spots caused by the oil had not left the fruit as late as August 1. Such spotting frequently occurs on citrus following oil sprays, but if not too severe it usually disappears long before the fruit takes on the color of maturity.

Extensive field tests were made with kaolin emulsion through the entire season of 1923. Many thousand gallons were used in combination with various strengths of Bordeaux mixture during the scab and melanose spraying seasons, and in every case equally satisfactory results were obtained as with soap emulsion, and no injury followed. It was also used extensively in combination with lime-sulphur solution and dry lime sulphur during late June and early July to kill scale insects and rust mites following Bordeaux oil emulsion applied for scab or melanose control.

As a general thing it gave satisfactory results in controlling scale insects and rust mites, but in some cases considerable injury followed. The kaolin emulsion alone has never caused any damage different in appearance or extent from that following soap emulsion applied under similar conditions, and in only a few instances has lime-sulphur solution caused damage. There seems to be a greater liability to damage when an emulsion is combined with lime-sulphur solution than when the two are applied separately.

Fuller's earth was used with excellent results by a large fruit company on several acres of oranges and grapefruit during 1923 and on about 400 acres during 1924. It was used with "hard" or "sulphur" water from deep wells, and it mixed better than any emulsion ever before used by this company. They used a heavy oil diluted to $1\frac{1}{3}$ per cent and got some oil burn. The oil concentration was reduced to 1 per cent and still some burn developed. They then added 2 pounds of hydrated lime to each 50 gallons of diluted spray and thereby practically eliminated the spray injury.

KAOLIN AND FULLER'S EARTH EMULSIONS WITH CALCIUM CASEINATE

Both kaolin and fuller's-earth emulsions when applied on citrus foliage have the appearance of not spreading very well, but, as will be shown later, this appearance is entirely deceptive. To overcome this apparent objection, several experiments with calcium caseinate were tried. On May 23, 1923, six orange trees were sprayed, using kaolin emulsion $1\frac{1}{3}$ per cent in the diluted spray and calcium caseinate at the rate of $1\frac{1}{2}$ pounds to each 50 gallons of water. This spray spread over the leaves and fruit uniformly and did not collect in drops on the fruit except on the lower side. On August 27 two 50-gallon tanks of kaolin emulsion and two tanks of fuller's-earth

emulsion were applied on budded orange trees. To one tank of each was added 8 ounces of calcium caseinate, one load of each not having this material. The calcium caseinate made the spray spread better when it was being applied and it was a much smoother material; but on the following day there could be observed no difference in the way the respective sprays had spread and dried on the foliage. Apparently there was just as even a film of oil on one plot as on the other. From this test there is some question whether it is profitable to add calcium caseinate to kaolin emulsion.

KAOLIN AND FULLER'S EARTH LIME-SULPHUR OIL EMULSION

Kaolin lime-sulphur oil emulsion was used on August 27 to spray some young orange trees, at the rate of $1\frac{1}{2}$ gallons to each 50 gallons of spray. This gave a 1-50 lime-sulphur solution and a 1 per cent oil, the normal dilution for both when applied separately. These three 50-gallon loads mixed very satisfactorily and came out of the machine nicely. The spray seemed to spread over the fruit and foliage evenly, but did not have the smooth appearance of the soap emulsions. No injury was apparent 24 hours after spraying, but 8 days later it was observed that considerable shedding of foliage had taken place. The foliage shed, however, consisted mainly of old leaves. Some fruit also was damaged on the upper surfaces. At first this appeared to be excessive, but it is doubtful that more than 50 fruits were damaged on the entire plot which received the 150 gallons of material. The injury, however, was greater than good grove practice would allow.

Two loads of 50 gallons each of fuller's earth lime-sulphur oil emulsion were sprayed on oranges on August 27, 1923, one with and one without the addition of calcium caseinate. This made the dilutions of the lime-sulphur and oil emulsion the same as is frequently used in grove practice.

No injury was noticed at the expiration of 24 hours, but 8 days after spraying defoliation was very severe, much of the fruit had been burned on the side toward the sun and many limbs one-half inch in diameter and smaller were dead. The injury resembled that which usually follows applications of sulphonated oil on citrus trees and fruit. After such severe injury it was thought best to discontinue further work on this formula. Fuller's earth and kaolin emulsion (made with water instead of lime-sulphur solution) were used on the same day without any injurious results.

DIRECTIONS FOR MAKING KAOLIN OR FULLER'S EMULSION IN LARGE QUANTITIES

To make 3 barrels of emulsion put 50 gallons of water in the clean tank of a power sprayer and add 133 pounds of kaolin or fuller's earth to the water; let the water act upon the clay for one-half hour, or until it is completely saturated. Up to this point no stirring whatever should be done, as stirring retards complete dispersion of the kaolin by forming an apparently impervious coating around the larger lumps. Add 100 gallons of oil of the desired consistency. As soon as the clay is completely dispersed start the agitator and pump. The mixture should be pumped into another container and then pumped back again, or pumped directly back into the original tank

until the emulsion becomes thick, when it may be pumped into storage barrels. A good emulsion looks much like a firm mayonnaise salad dressing.

This emulsion has been made many times in 50-gallon and several times in 150-gallon lots by running the mixture twice through the pump of a power spraying outfit.

SUMMARY

Kaolin, fuller's earth, and other colloidal clays may be used in lieu of soap in making emulsions of mineral lubricating oils for spraying citrus trees. These emulsions are about as effective against insect pests as are the soap emulsions, and no more likely to cause spray burn. The main value, perhaps, in these clay emulsions is that they will mix with any water or desired spray combination without special treatment, are easily made, keep indefinitely, and cost much less than soap emulsion, both for material and for labor. The kaolin used for the emulsion should not cost more than from one-sixth to one-ninth as much as the soap, and fuller's earth is cheaper than kaolin. The principal objection to these emulsions is that they are a semipaste rather than a fluid.

RELATION BETWEEN CERTAIN HERITABLE PROPERTIES OF WHEAT AND THEIR CAPACITY TO INCREASE PROTEIN CONTENT OF GRAIN¹

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Table I gives the relation of two heritable characters of wheat with the percentage of increase in protein of the grain produced by an application of nitrogen supplied relatively late in the growth period of the plants. Explanation of the values of the data is as follows:

Column A gives the maximum per cent of gain in protein assumed to be due to application of nitrogen to the plant. The values were obtained by dividing the per cent protein in the grain of cultures that received nitrogen when 90 days old by that contained in the cultures which produced the lowest per cent of protein. The lowest values were obtained either from the cultures that did not receive nitrogen or those that received it at the time of planting. (That nitrogen supplied to wheat in pot cultures, or in the field at the time of planting does, in some cases, result in a product lower in protein than would obtain if this material were not supplied, has been observed by some other investigators. As the explanation for this phenomenon would be aside the subject matter of this article, it will not be considered in this paper.)

Column B gives the per cent grain to total dry matter. These values represent differences considered as heritable among the varieties. (While the characters in columns B and C are considered by agronomists to be heritable and represent distinctions among varieties, it is known that the values of these characters may be changed by environmental conditions. Varieties showing marked differentiations, however, usually maintain approximately their relative order for these characters even under diverse conditions. However, it is necessary, in a study of this kind, to appreciate that the relative values of any of the characters may change.²)

Column C represents the relative capacity of the varieties to ripen after nitrogen was applied, which factor may also be considered as genetic. The values were obtained by dividing the number of days required for ripening of plants which did not receive nitrogen by the number of days before ripening that nitrogen was applied to the cultures which produced the grain that had the highest per cent protein.

Column B differs from column A in that the order for Sonora and Fulcaster for third and fourth places, and for Hard Federation and Dart's Imperial for fifth and sixth places, are interchanged. When columns A and C are compared, it is to be noted that the varieties for fifth and seventh places are interchanged.

¹ Received for publication Sept. 19, 1924; issued September, 1925.

² GERICKE, W. F. SOME EFFECTS OF PHYSIOLOGICAL CONDITIONS ON GENETIC CHARACTERS OF WHEAT. *Amer. Jour. Bot.* 10: 275-277. 1923.

TABLE I.—*Relation between two heritable properties of wheat varieties and their capacity to increase the protein content of grain*

A		B		C	
Capacity to increase in protein		Ratio of grain to total weight		Capacity to ripen after treatment	
Bunyip.....	1.738	Bunyip.....	44.5	Bunyip.....	3.00
Cedar.....	1.635	Cedar.....	40.5	Cedar.....	2.76
Fulcaster.....	1.625	Sonora.....	38.9	Fulcaster.....	2.36
Sonora.....	1.576	Fulcaster.....	37.7	Sonora.....	2.29
Dart's Imperial.....	1.559	Hard Federation.....	35.1	Early Baart.....	2.22
Hard Federation.....	1.500	Dart's Imperial.....	32.8	Hard Federation.....	2.21
Early Baart.....	1.498	Early Baart.....	32.7	Dart's Imperial.....	2.06
White Australian.....	1.395	White Australian.....	32.6	White Australian.....	1.95
Marquis ^a	1.350	Marquis.....	30.2	Marquis.....	1.88

^a For some reason, Marquis did not grow well. There was considerable mortality among the cultures. It is probable, therefore, that the order given it does not indicate its true value among these varieties.

The way these varieties (which represent a range of very early to very late wheats) fall in order of correlation at once suggests causative relations in some of their heritable properties and in the capacity of wheat varieties to produce high-protein grain. Owing to the fact that the protein content of grain can be increased by merely supplying nitrogen to the plants at certain phases of their growth, it follows that the genetic characters of varieties which are correlated to the capacity of the plants to increase the protein content of the grain must be agencies which influenced the physiological processes determining the protein content of wheat. As this factor obviously bears on the relative rate of intake of nitrogen to that of other processes of growth, it follows that the effect of these genetic properties on the protein content of wheat must influence the rate of absorption and utilization of nitrogen by the plants.

The ratio of grain to total dry matter is an expression of the relative power of varieties to utilize absorbed material, including nitrogen, for grain production. However, as the per cent of protein in grain of any variety is determined by the amount of nitrogen those plants utilize for grain, and by the amount of grain produced, it follows that any change in this value must be due to changes in either or both factors. Whether wheat varieties which have the genetic property to produce high values of grain to total dry weight also possess correspondingly great capacity to produce high-protein grain when physiological conditions supervene which make this possible, has apparently not been determined. The fact of correlation between these properties, as shown by the data, is evidence that varieties which have great efficiency for production of grain to total dry weight likewise have correspondingly great capacity to augment the protein content of the grain above the minimum for that variety. This, of course, does not mean that those varieties characterized by high percentage of grain to total dry weight invariably produce higher protein grain *per se* than do those varieties that do not have this genetic feature. The ratio of grain to total dry matter is only a measure of the capacity of the plants to increase the nitrogen in the grain and can not by itself determine what the absolute protein content of the grain will be. Furthermore, this property is also an expression of the degree of fluctuation to which the variety is subject.

Whether or not high-protein grain will result from the capacity of any variety of wheat to produce high percentage grain to total

dry weight, granted that the proper physiological conditions prevail, depends largely on what the minimum for that variety happens to be. If, for example, two different varieties each having the same percentage of grain to total dry weight, and, consequently, equal capacity to increase the protein content of their grain, having 6 and 8 per cent protein, respectively, as the minimum, would, as a result of treatment, produce grain that would vary proportionally in protein content. But the increase in one case, say it was 50 per cent, would be such as to retain for that product the character of soft wheat, while equal per cent increase in the other variety would result in grain belonging to the hard wheat class; 9 and 12 per cent, respectively, would be the per cent protein of these two varieties.

In the correlation between the order of ripening of the varieties after nitrogen was supplied, and that of the gain in per cent protein of the grain, is evidence that the genetic property which determines how quickly any wheat variety matures likewise expresses the relative capacity of that variety to increase the protein in grain. Whether any variety produces high-protein grain is, however, not determined by the mere property of earliness but by the physiological condition already defined, namely, the relative rate of the absorption of nitrogen, which, in turn, is dependent on the supply available to the plants. Thus if varieties requiring different numbers of days are to absorb and utilize a given limited amount of nitrogen supplied to the culture 90 days after planting, it is obvious that those varieties which utilize this material in the least number of days have a higher rate for that process, or processes, than have varieties which require a greater number of days. It is in this relation that the genetic property of earliness bears on the per cent protein in grain. It affects the relative rate of intake of nitrogen by the plants. Therefore, wheats that are early by heritable property are physiologically better adapted to produce higher-protein grain than are late wheats. This, however, in turn, does not mean that late varieties are necessarily low-protein wheats. On the contrary, many are relatively high in protein. If sufficient nitrogen is available to the plants for a period of three to six weeks during certain phases of their growth (differing with the varieties), which includes the latter part of the "shooting" period for the culms, the period of heading, and the early part of the period of filling of the mold, then high-protein grain obtains. On the other hand, even though the soil does not contain as much nitrogen as the plants could absorb (conditions that usually prevail in wheat-growing sections), this does not necessarily infer the production of low-protein wheat. Other compensating factors, such as that related to ratio of grain to total dry weight, can preclude the performance of results of any specific factor.

As the protein content of wheat can be markedly affected by manipulation of the supply of nitrogen in the growth media, so climate, season, and soil, through their influence on the rate of supply of nitrogen to the plants must affect the protein content of the grain. In some regions climate and seasonal changes on varietal characters will have more pronounced influence on the quality of grain than in other sections. Likewise the effect of properties of soil on the protein content of grain may be markedly altered by the influence of climate and season. It therefore follows that while the

rate of supply of nitrogen to the growing plants determines the protein content of grain, nevertheless many factors may affect the rate of supply of nitrogen to the plants. Thus the methods of culture to be employed which will effect the desired end will vary with, and depend on, conditions that prevail in wheat-growing areas. Undoubtedly the quality of wheat could be markedly improved in many places if cultural methods were used that would provide the production of sufficient nitrogen during a portion of the latter part of the growth period of the plants. Application of nitrate to partially matured plants in fields where the grain is usually low in protein is, in most cases, impractical. Furthermore, success of this method would be conditioned by ample rainfall to make the salt available, and this is usually precluded by the character of the season.

Thus it appears that the most promising method would be one that would utilize the character of soil and particularly that fraction which is organic matter to act both as a storehouse for and a regulator of the supply of available nitrogen. Certain methods of soil management and crop rotation could be instituted that would undoubtedly affect the rate of supply of nitrogen for any particular year in which wheat is to be grown on any given piece of land. Manures which nitrify relatively slowly presumably could be used to advantage in the production of high-protein wheat.

But aside from the improvement of the quality of wheat directly through nutrition, methods of selection and breeding for high-protein varieties undoubtedly must be continually employed. However, because of the failure to recognize, first, the underlying physiological cause for differences and variation in the protein content of wheat, and second, the relation of genetic characters to the physiological cause, the method for improvement of wheat by selection has been rendered quite tedious. It has kept workers in the dark as to the essential factors that determine the desired results. It is, therefore, thought that the relationships here shown to exist between certain heritable characters of varieties and their capacity to produce high-protein grain will be helpful to plant breeders and agronomists in obtaining such varieties of wheats as will produce high-protein grain even under relatively unfavorable soil and climatic conditions.

SPREADERS FOR SPRAY MATERIALS, AND THE RELATION OF SURFACE TENSION OF SOLUTIONS TO THEIR SPREADING QUALITIES¹

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INTRODUCTION

For many years efforts have been made to improve the spreading and adhering qualities of spray materials by the addition of some harmless substance that would cause the spray to form a continuous, even film over the plant surface covered. At present our greatest need in orchard practice, aside from the development of certain more effective sprays, is to overcome the difficulty attending the application of sprays in which the surface can not properly and easily be covered.

The continual increase in severity of infestation and the appearance of some insect pest or fungous disease into a new section of the country emphasizes the necessity of either heavier and more careful applications of the poison whereby all of the plant surface will be covered, or the use of a more effective spray. Since most of our important sprays are effective to the extent that fair control has been obtained with them when care is exercised to obtain complete coverage, it is obvious that the main factor contributing to the ravages caused by insect pests and fungous diseases is the inability to cover with comparative ease the entire surface. If the addition of a spreader to a spray improves the covering and assists in more easily obtaining a protective coating of the poison on those surfaces to which the sprays adhere with difficulty, then an important advance has been made in spraying.

An ideal spreader should cause a continuous film of the poison to be deposited on the surface covered instead of collecting in drops, it should increase adherence, it should not react chemically with the poison to form by-products that would cause foliage injury, it should not diminish the toxic properties of the poison, and it should not cost too much. The difficulty in selecting a spreader with these requirements is increased by the fact that the plant may have several different kinds of surfaces. For example, the apple tree has the smooth upper leaf surface, the hairy under leaf surface, and the waxy surface of the partially developed fruit; and in addition it may have the smooth bark and the wrinkled bark of spurs and the young and old leaves which may offer resistance in various ways to the spray. Ruth and Kelley² state that the behavior of the surfaces toward sprays changes very rapidly, in some cases markedly within a week or 10 days. It may be impossible to find a spreader that will fulfill all of the requirements. Investigations, however, may reveal materials or combinations of materials that will prove beneficial on certain plants at different periods of growth.

¹ Received for publication Aug. 24, 1924; issued September, 1925.

² RUTH, W. A., and KELLEY, V. W. RECENT ADVANCES IN SPRAYING. Unpublished data. 1922.

The present investigation was undertaken (1) to make a study of the known spreader materials and to search for others that may be more practical; (2) to correlate the surface-tension values of various materials with their spreading properties, and to learn more regarding the fundamental causes of spreading; and (3) to devise a laboratory method whereby the approximate spreading properties of a substance may be estimated.

REVIEW OF LITERATURE

Previous investigations on the spreading properties of different substances consider a wide variety of materials. A very complete bibliography of the literature is given by Moore (7)³ in his general discussion of the subject, and the review will not be repeated here. Summarized briefly, previous investigations have shown that various colloidal substances are most effective in modifying the spray mixture to make it spread over various surfaces and form a continuous film on the objects sprayed. Among those colloids, casein, soap, glue, saponin, and flour have been found adaptable under certain conditions. Other organic materials such as creosote, eugenol, and cresylic acid are reported as having spreading properties on waxy surfaces, but their high cost and other objectionable features make them impracticable for use.

The theory of spreading as applied to spray materials is presented in recent publications by Cooper and Nuttall (1) and by Moore (7). They elaborate upon the principles of wetting or spreading as first established by Quincke (8) in 1877. In a study of the capillary rise of liquids in tubes of different materials the latter formulated the conditions necessary for wetting or spreading to occur. These conditions as applied to spreading provided that in order to state definitely whether a liquid will wet or spread on a solid (or another liquid) it is necessary to know the values for the surface tension of the liquid and of the solid and the interfacial tension of the liquid to the solid. Spreading of a certain liquid or spreader solution would then occur if the surface tension of the solid or surface sprayed is greater than the sum of the surface tension of the liquid and the interfacial tension of the liquid to the solid. The magnitude of the inequality would indicate good or poor spreading of the liquid on the solid. Since, however, the surface tension of the solid—if a solid has surface tension—is indeterminate, it would be impossible to obtain the actual values necessary to ascertain whether a substance would be a good spreader for a specific surface, as, for instance, the apple leaf or fruit. If approximate values could be obtained by substituting a liquid for the solid, as, for example, an oil for the waxy surface of fruit or leaf, the probable spreading properties of a given substance might be estimated. This was accomplished successfully by Cooper and Nuttall (1) in their evaluation of the wetting power of various stock dips. Reference will be made to this procedure later.

From the preceding it is apparent that any spray solution to spread well should have both a low surface tension and a low interfacial tension to the solid or surface sprayed, as the sum of these values must be less than the surface tension of the surface sprayed. If a

³ Reference is made by numbers (italic) to "Literature cited," p. 81.

spray does not possess these requirements it may be modified by the addition of some substance that reduces the tension values. The question then arises, what substances will reduce the surface tension of a solution. It is well known that adsorption of a solution at the surface layer will cause a reduction in the surface tension. The same is true regarding adsorption at the interface of a liquid and solid. It then becomes a search for those substances that are adsorbed at the liquid-air interface and the liquid-solid interface. Moore (7), in answer to the question regarding those substances that are positively adsorbed in the surface layer or at the interface of a liquid and solid, applies the theory of orientation of molecules at the surface or interface. From the work of Harkins, Brown, and Davies (3), of Harkins, Davies, and Clark (4), and of Langmuir (6), Moore suggests that those substances that contain active polar groups which render them soluble in water and inactive groups that cause adsorption at the surface and thus reduce the surface tension should augment the spreading properties of the solute. Then if the inactive group is similar to the surface (of the plant) to be covered or soluble in it, spreading should result.

Since the amount of adsorption in the surface layer of a solute and at the liquid-solid interface indicates, to a certain extent, the spreading power of the solution, a measurement of the surface and the interfacial tension values should disclose its possibilities as a spreader.

EXPERIMENTAL DATA

In order to obtain more complete data on surface adsorption of various materials, surface-tension determinations of numerous substances were made. The method employed was mechanical and rapid. The Du Nous instrument was used at first, but a new apparatus devised by Fahrenwald (2) was found more adaptable for the work at hand. This apparatus measures the surface tension of a solution by the film method. An accuracy of 0.1 dyne-cm. is claimed for the instrument.

All measurements were made at 25° C. The time necessary for static equilibrium to be established was found to vary appreciably with the different substances. In order to approximate the time it takes for a drop of spray to leave the nozzle of the spray gun and to settle on the surface of the plant, about three minutes were allowed for the development of partial static tension before the measurement was recorded. The results given in Table I report the observations made on part of the miscellaneous substances studied.

In order to correlate the surface-tension results with the actual spreading qualities of a substance, several kinds of leaves and half-grown apples were sprayed with similar solutions on which surface-tension observation had been made. The leaves and fruit were sprayed with an atomizer, and the degree of spreading as observed was recorded. The degree of spreading designated by the term "good" indicates the formation of an even film covering the entire surface, "medium" signifies that small portions of the surface drained dry, "poor" indicates very slight spreading, and "none" means spreading on the surface similar to distilled water. The degree of spreading of various substances dissolved or suspended in distilled water at the concentration indicated is given in Table II.

TABLE I.—Surface tension of miscellaneous substances in solution

Material	Con- centra- tion	Surface tension	Material	Con- centra- tion	Surface tension
	Per cent	Dynes per cm.		Per cent	Dynes per cm.
Water		72.8	Tannin	1.0	48.4
Sodium palmitate	0.1	31.2	Do	.1	57.9
Do	.01	40.5	Do	.01	71.5
Do	.001	50.7	Gum arabic	1.0	72.0
Sodium stearate	.1	34.6	Do	.1	72.1
Do	.01	39.4	Do	.01	72.1
Do	.001	51.0	Glycerine	1.0	71.5
Sodium oleate	.1	24.0	Do	.1	71.5
Do	.01	31.7	Creosote (meta)	1.0	48.0
Do	.001	49.0	Do	.1	69.5
Potassium oleate	.1	25.1	Creosote (beechwood)	.5	43.6
Do	.01	32.0	Do	.05	67.2
Do	.001	54.5	Cresol	1.0	41.0
Soap, Castile	.1	23.2	Do	.1	49.5
Do	.01	31.9	Resorcin	1.0	55.9
Soap, Ivory	.1	25.6	Do	.1	69.2
Do	.01	35.4	Phloroglucine	1.0	62.9
Do	.001	48.3	Do	.1	71.5
Soap, linseed oil (jell)	.1	26.7	Guaiacol	1.0	45.6
Do	.01	37.8	Do	.1	70.0
Do	.001	49.5	Pyrogallol	1.0	70.8
Mineral oil emulsion No. 1	4.0	26.9	Do	.1	71.4
Do	2.0	33.1	Hydroquinine	1.0	71.5
Do	.1	50.6	Oil of cloves (in 10 per cent al- cohol)	1.0	39.4
Mineral oil emulsion No. 2	4.0	38.1	Do	.1	44.2
Do	2.0	42.3	Oil of Eucalyptus (in 10 per cent alcohol)	1.0	32.0
Do	.1	55.9	Do	.1	44.3
Casein (in saturated Ca (OH) ₂)	1.0	51.4	Oil of cedar	.1	42.1
Do	.1	51.7	Oil of cedar (in 10 per cent alcohol)	.1	42.1
Do	.01	53.8	Oil of cottonseed	.1	47.4
Casein (plus 1 per cent Ca (OH) ₂)	1.0	51.4	Sodium oleate 0.01 per cent + casein (NaHCO ₃)	.01	30.4
Do	.1	52.0	Sodium oleate + 0.001 per cent casein	.001	51.3
Do	.01	54.0	Sodium oleate 0.01 per cent + casein	.01	.338
Casein (plus 0.1 per cent Na- HCO ₃)	1.0	43.0	Sodium oleate 0.001 + casein (Na- HCO ₃)	.001	50.0
Do	.1	47.2	Oil of cloves 0.1 per cent + sodium oleate	.01	37.8
Do	.01	49.8	Oil of cloves 0.1 per cent + glycerin	.1	44.3
Milk, skim (on dry basis)	.1	48.0	Oil of cottonseed 0.1 per cent + sodium oleate	.01	38.7
Do	.01	50.1			
Gelatin (purified)	1.0	51.3			
Do	.1	56.5			
Do	.01	62.2			
Glue (commercial)	1.0	46.0			
Do	.1	56.2			
Do	.01	64.8			
Saponin bark	1.0	41.3			
Do	.1	41.6			
Do	.03	50.0			

A comparison of the surface-tension values of the different substances in solution with their spreading qualities, shows that there is no consistent relation between them. Those substances that spread well have a much lower-surface tension than water, but other solutions having even lower values do not show as good spreading qualities. For example, a 0.01 per cent solution of casein, albumin, or saponin spreads well on all of the surfaces tested. On the other hand, the soaps at 0.01 per cent concentration did not spread any better than water, although the surface tension was far below the values for casein, albumin, or saponin. It is evident from these results that there are other influencing factors besides surface ad-sorption that affect the spreading qualities of a solution.

The other variable—namely, the tension at the interface of liquid-solid—is indeterminate. However, it was thought that by substituting an oil for the solid, as suggested by Cooper and Nuttall, (1) and making surface and interfacial determinations, that some interesting data would be obtained. Accordingly, a mineral oil having a specific

TABLE II.—*The spreading properties of various substances on fruit-tree leaves and apple fruit*

Substance	Concentration	Apple leaves	Pear leaves	Prune leaves	Apple fruit
	<i>Per cent</i>				
Sodium palmitate.....	1.0	Good.....	Good.....	Good.....	Good.....
Do.....	.1	Medium.....	Poor.....	Poor.....	Medium.....
Do.....	.01	None.....	None.....	None.....	None.....
Sodium stearate.....	1.0	Good.....	Good.....	Good.....	Good.....
Do.....	.1	Medium.....	Poor.....	Poor.....	Medium.....
Do.....	.01	None.....	None.....	None.....	None.....
Sodium oleate.....	1.0	Poor.....	do.....	Poor.....	Poor.....
Do.....	.1	None.....	do.....	None.....	None.....
Do.....	.01	do.....	do.....	do.....	Do.....
Potassium oleate.....	1.0	Poor.....	do.....	Poor.....	Poor.....
Do.....	.1	None.....	do.....	None.....	None.....
Do.....	.01	do.....	do.....	do.....	Do.....
Soap, Ivory.....	1.0	Good.....	Good.....	Good.....	Good.....
Do.....	.1	None.....	None.....	None.....	None.....
Linseed-oil soap.....	.1	Medium.....	Poor.....	Medium.....	Poor.....
Casein (in Ca(OH) ₂ sol.).....	.01	Good.....	Good.....	Good.....	Good.....
Casein (in NaHCO ₃).....	.01	do.....	do.....	do.....	Do.....
Milk, skim (fresh).....	1.0	do.....	do.....	do.....	Do.....
Milk, skim (24 hours old).....	1.0	None.....	None.....	None.....	None.....
Milk, skim, plus Ca(OH) ₂	1.0	Good.....	Good.....	Good.....	Good.....
Gelatin.....	.1	do.....	do.....	do.....	Do.....
Do.....	.01	Medium.....	None.....	None.....	None.....
Glue.....	.1	Good.....	Good.....	Good.....	Good.....
Saponin bark.....	1.0	do.....	do.....	do.....	Do.....
Do.....	.1	Medium.....	None.....	Poor.....	Poor.....
Tannin.....	.1	do.....	Poor.....	Medium.....	Do.....
Gum arabic.....	1.0	None.....	None.....	None.....	None.....
Pepsin.....	.1	Good.....	Medium.....	Medium.....	Medium.....
Flour, hard wheat.....	.1	do.....	Good.....	Good.....	Good.....
Do.....	.01	Medium.....	Poor.....	Medium.....	Poor.....
Agar agar.....	.1	do.....	do.....	Poor.....	Medium.....
Do.....	.01	do.....	do.....	do.....	Poor.....
Blood (water extract).....	1.0	Good.....	Good.....	Good.....	Good.....
Do.....	.1	Medium.....	Poor.....	Poor.....	Medium.....
Albumin.....	.1	Good.....	Good.....	Good.....	Good.....
Do.....	.01	do.....	do.....	do.....	Do.....
Red clover hay (extract).....	2.0	do.....	do.....	do.....	Good.....
Do.....	.2	Medium.....	Poor.....	Poor.....	None.....
Vetch hay (extract).....	2.0	Good.....	Good.....	Good.....	Good.....
Alfalfa hay (extract).....	2.0	do.....	do.....	do.....	Do.....
Creosote oil.....	1.0	None.....	None.....	None.....	None.....
Creosote, beechwood.....	1.0	do.....	do.....	do.....	Do.....
Cresol.....	.1	Medium.....	do.....	do.....	Poor.....
Evgenol.....	.5	do.....	do.....	Poor.....	None.....
Phloroglucine.....	.1	Poor.....	do.....	None.....	Do.....
Guaiacol.....	.1	do.....	do.....	do.....	Do.....
Resorcin.....	.1	do.....	do.....	do.....	Do.....
Oil of cottonseed.....	.1	Medium.....	do.....	Poor.....	Do.....
Oil of cedar.....	.1	do.....	do.....	do.....	Do.....
Oil of pears.....	.1	None.....	do.....	None.....	Do.....

gravity of 0.869 at 20° C. was selected and observations made on the interfacial tension of many substances to the oil. The surface tension of the oil was 30.4 dynes per centimeter at 25°.

The apparatus devised by Fahrenwald (2) and used for these surface-tension determinations was used also for interfacial determinations. It is claimed that this is the only instrument made that will give fairly true static interfacial tension values. This is important in that when a spray issues from the nozzle of the spray gun in drops of various sizes it establishes, at least partially, static equilibrium by the time it comes into contact with and settles upon the plant surface. After contact, static equilibrium is established at the interface of spray solution to plant surface, quickly or slowly, depending upon the spreader solution used. Measurements were recorded about 3 minutes after the oil was introduced upon the surface of the spreader solution. Table III gives the results of the observations made at 25° C.

TABLE III.—Interfacial tension between paraffin oil and miscellaneous substances in solution

Material	Con- centra- tion	Inter- facial ten- sion	Material	Con- centra- tion	Inter- facial ten- sion
	Per cent	Dynes per cm.		Per cent	Dynes per cm.
Water		24.4	Casein (in 0.05 per cent NaHCO ₃)	0.1	9.2
Sodium palmate	0.1	11.0	Do	.01	11.5
Do	.01	19.7	Milk, skim (on dry basis)	1.0	9.4
Do	.001	22.1	Do	.1	10.1
Sodium stearate	.1	8.2	Do	.01	11.9
Do	.01	19.2	Gelatin (purified)	1.0	10.8
Do	.001	22.1	Do	.1	14.6
Sodium oleate	.1	3.8	Do	.01	17.4
Do	.01	17.1	Glue (commercial)	1.0	12.2
Do	.001	20.8	Do	.1	15.3
Potassium oleate	.1	5.5	Do	.01	17.5
Do	.01	18.1	Saponin (bark extracted with water)	1.0	5.0
Do	.001	21.4	Do	.1	5.4
Soap, Castile	.1	6.1	Do	.03	9.3
Do	.01	16.6	Tannin	1.0	10.1
Soap, Ivory	.1	10.2	Do	.1	18.0
Do	.01	18.6	Do	.01	19.3
Soap, linseed oil	.1	7.1	Gum arabic	1.0	15.4
Do	.01	12.6	Do	.1	18.6
Mineral oil emulsion No. 1	4.0	2.5	Do	.01	20.2
Do	2.0	6.0	Creosote (meta)	.1	20.2
Do	1.0	10.1	Creosote (beechwood)	.1	20.8
Do	.1	18.1	Cresol	.1	17.1
Mineral oil emulsion No. 2	4.0	7.5	Resorcin	.1	21.0
Do	2.0	10.6	Guaiacol	.1	20.8
Do	.1	18.1	Pyrogallol	.1	21.1
Casein (in saturated lime water)	1.0	8.5	Phlorglucine	.1	20.2
Do	.1	8.6	Evgenol	.1	19.0
Do	.01	11.5	Oil of cloves	.1	16.5
Casein (in 1 per cent Ca(HO) ₂ suspension)	1.0	6.7	Oil of Eucalyptus	.1	16.0
Do	.1	8.1	Oil of cedar	.1	14.4
Do	.01	11.2	Oil of cedar in 10 per cent alcohol	.1	19.9
Casein (in 0.05 per cent NaHCO ₃)	1.0	8.3	Oil of pears (amyl acetate)	.1	21.3

Correlating the results in Table III with the actual spreading properties of the different substances as observed in Table II, it is to be noted that the interfacial tension values, where oil is substituted as the solid, are not proportional to the spreading properties of the various materials. If conditions favorable to spreading are expressed in equation form, then:

$$A_t > B_t + A_t B_t$$

in which A_t is the surface tension of the solid, or surface to be covered; B_t , the surface tension of the liquid or spreader solution; and $A_t B_t$, the interfacial tension between the liquid and solid. By substituting the values given in Tables I and III one finds that negative results are obtained for most of the substances tested. This indicates that there is not complete wetting or spreading of the different substances on the oil used. As an example, the surface tension of the oil (A_t) was 30.4 dynes-cm.; the surface tension of a 1.0 per cent solution of sodium palmitate (B_t) was found to be 31.2 dynes-cm.; the tension at the solute-oil interface ($A_t B_t$) was 11.4 dynes-cm. Substituting these values in the inequality one obtains -12.2 . Although good spreading was observed on the solid or surface tested in Table II, the negative value, -12.2 , indicates the opposite on the oil as a solid. Other oils, both mineral and vegetable, were also used, but similar results were obtained. The oils used, therefore, can not be substituted for waxy or other surfaces of the plant to

obtain values whereby the spreading qualities of a solute may be estimated by substituting the surface tension values in the inequality just given.

Although specific deduction can not be made from the surface and interfacial tension values regarding the spreading properties of various solutes, possibilities may be suggested, especially if the chemical composition is taken into consideration. Low values indicate in general surface adsorption and all substances tested and showing this phenomenon have certain spreading qualities. There are probably other influencing factors that affect spreading. Such factors as the solvent action of the spreaders on the surface to be covered, the rapidity with which static equilibrium is established, and the formation of plastic solids at the interface may benefit or reduce spreading.

Further examination of the observations reported in Table II discloses why it is difficult to generalize on the spreading qualities of any particular substance. It will be seen that some materials at a certain dilution spread well on apple leaves, and "medium" or "poor" on pears or prune leaves. Other variations are shown as "medium" spread on apple and prune leaves, while only "poor" spreading is obtained on pear leaves and apple fruit. It was also noticed that the age and exposure of the leaf to the sun's rays influenced the degree of spreading. For example, a solute at minimum concentration would spread nicely on a young apple leaf, while barely perceptible spreading would occur on an old leaf taken from the exposed side of the tree. In many cases, however, a higher concentration of the spreader gave better results. Ruth and Kelley⁴ likewise observed that it was very difficult to wet certain areas of a tree, that differences in surfaces existed, and that the surfaces changed throughout the growing season. This change in surface, whether leaf or fruit, probably accounts for contradictory results obtained by different workers using the same kind of spreader. Under these circumstances it would be futile to conclude from laboratory tests that a substance would spread on all surfaces.

In a search for better and more practical spreaders many substances and combinations of two or more materials were studied. As negative results were obtained, many of them are not reported in the tables. The materials that aided best in producing a film over the surfaces tested may be divided into two classes, namely, the soaps and the substances containing water soluble or colloidal solutions of proteins. In general, the soaps required much higher concentration than the soluble proteins to produce equal spreading on most of the surfaces tested. Contrary to Moore's opinion that spreading of casein would not occur unless sufficient force was applied to penetrate the wax covering of the cabbage leaf, a good film was formed with most of the soluble protein materials, when sprayed to the drenching point. The force necessary depended upon the concentration of the protein, being greater for the lower concentrations and less at the higher concentrations. This indicates a flattening of the droplets by force until they cohere to form a continuous film instead of penetrating the waxy surface. The same holds true for the waxy covering of the apple.

⁴ RUTH, W. A., and KELLEY, V. W. RECENT ADVANCES IN SPRAYING. Unpublished data 1922.

When it was observed that solutions containing soluble proteins spread well on most surfaces, the study of this class of material was extended to include many other similar substances. Among these, albumin, pepsin, hot water extracts of dried blood and of sage, dried skim milk powder and hot water extracts of such hays as clover, vetch, alfalfa, and dried grasses, spread well on all surfaces tested, even at low concentrations. This further substantiates the opinion that the soluble protein present is the active spreading agent. It is probable also that the active elements in the "Irish moss" used successfully by both Issleib (5) and Stearns (9) were soluble protein substances.

The use of spreaders for general orchard sprays has become more prevalent during the last few years. The development of the commercial casein-lime product has contributed mainly to this increase. Questions regarding the advisability of using spreaders, the kind to use, and the concentration that will give best results can be answered satisfactorily only when all conditions are taken into consideration. The cost is perhaps the most important point to consider. If the cost is low, spreaders may be used advantageously in most sprays, but if they are as high as present prices that prevail for the commercial casein-lime it should be used only for those sprays that unquestionably are improved by it and when beneficial results are obvious. It does not seem justifiable under average conditions to pay as much for the spreader as for the spray material itself, where comparative results indicate only a slight advantage in control. Under abnormal conditions and in parts of the country where severe epidemics of disease or insects prevail, their general use may be justified. The use of spreaders also in the last two cover sprays for apples and pears may be advisable in order to obviate the necessity of wiping the fruit and to allow a more even color development.

If the cost of a spreader is low enough to allow a satisfactory profit to the grower, then the more general use of spreaders is advisable.

Considering price, skim milk and skim-milk products have proved to be excellent substitutes for the commercial casein-lime spreaders. Laboratory tests and limited field experiments indicate that when skim milk is used at the rate of 2 to 3 quarts to 100 gallons it spreads well over many surfaces such as apple, pear, prune, peach, cherry, and cabbage leaves, and apple and pear fruits. The dried product used in equivalent amounts, as at the rate of solids in skim milk or 2.5 ounces to the quart, and the partially dried clabbered milk such as is used for stock feed, give equally good results. It is necessary to add a few ounces of hydrated lime or slaked quicklime to each quart of sweet skim milk and larger amounts to the commercial stock foods in order to neutralize the acidity and to bring the proteins into colloidal solution. Unless the lime is added spreading will not result. Skim milk, the dried or condensed skim milk, and the clabbered product are brought into solution easily, and in this respect they are preferable to the commercial casein-lime that must be worked carefully to a paste before putting into the spray tank in order to get best results.

THE EFFECT OF SPREADERS ON THE AMOUNT OF POISON THAT ADHERES

When a good spreader is used the whole surface is wetted and an even coating of the spray is obtained. As the film is formed the excess runs off and gives the appearance of excessive drip. In order to learn whether less poison adheres to the surface when a spreader is used, a series of laboratory tests were made. Apple and pear leaves were sprayed with lead arsenate at several dilutions with and without a spreader in the spray. Milk, commercial casein-lime, and laboratory preparations of casein and hydrated lime were used as spreaders. The leaves were sprayed with an atomizer and suspended at a 45° angle to dry and to allow the excess to drain off. The surface areas of the leaves were measured by means of a planimeter. The amount of arsenic that adhered to 100 square inches of leaf surface was then determined. The results obtained are given in Table IV.

TABLE IV.—The amount of lead arsenate on 100 square inches of leaf surface

Lead arsenate concentration	Spreader treatment	Apple	Pear
		Mgm.	Mgm.
2 to 100.....	None.....	7.2	4.9
2 to 100.....	Casein 0.05 per cent in saturated Ca(OH) ₂ solution.....	6.8	6.2
2 to 100.....	Casein 0.05 per cent+Ca(OH) ₂ 0.08 per cent.....	6.1	5.6
4 to 100.....	None.....	12.0	16.0
4 to 100.....	Casein 0.10 per cent in saturated Ca(OH) ₂ solution.....	9.3	5.5
4 to 100.....	Casein 0.05 per cent+Ca(OH) ₂ 0.08 per cent.....	10.0	8.5
4 to 100.....	Skim milk 1.4 per cent+Ca(OH) ₂ 0.1 per cent.....	13.1	7.2
4 to 100.....	Whole milk 1.4 per cent+Ca(OH) ₂ 0.05 per cent.....	12.0	11.0
4 to 100.....	None.....	11.8	16.4
4 to 100.....	Casein 0.01 per cent+Ca(OH) ₂ 0.02 per cent.....	8.8	11.3
4 to 100.....	Casein 0.02 per cent+Ca(OH) ₂ 0.04 per cent.....	15.5	10.4
4 to 100.....	Casein 0.05 per cent+Ca(OH) ₂ 0.1 per cent.....	8.3	11.4
4 to 100.....	Commercial casein-lime 0.15 per cent.....	11.1	11.9
6 to 100.....	None.....	19.0	23.5
6 to 100.....	Casein 1 per cent in saturated Ca(OH) ₂ solution.....	10.2	13.3
6 to 100.....	Casein 0.02+Ca(OH) ₂ 0.04 per cent.....	21.2	12.6
6 to 100.....	Skim milk 2.0 per cent+Ca(OH) ₂ 0.04 per cent.....	19.5	12.4
6 to 100.....	Skim milk 2.0 per cent+Ca(OH) ₂ 0.08 per cent.....	17.0	11.0

It will be noted from the results given in Table IV that there is an appreciable variation which, perhaps, may be due to the age and size of leaves used and to the amount of lead arsenate that drained to the lower tip of the leaf. In general, however, it may be said that at all dilutions the apple leaves sprayed without a spreader show no more arsenic than where a spreader had been used. With the pear leaves, larger amounts of lead arsenate were found on the leaves sprayed without a spreader, except at the 2:100 dilution. However, the leaves sprayed without a spreader were covered in spots only, while where a spreader was used the coating of poison was an even film.

The amount of lead arsenate that adhered to the leaves was, for the most part, proportional to the concentration used. Approximately the same amount of lead arsenate was found on leaves where milk had been used in the spray as with the various forms of casein-lime.

In addition to the laboratory tests, samples of leaves sprayed under normal conditions in the orchard were collected and the amount of lead arsenate that adhered was determined. Several different

commercial brands of lead arsenate were used. The leaves were collected a few hours after spraying and the surface area measured as before. Table V gives the results obtained.

TABLE V.—*The amount of lead arsenate on 100 square inches of leaf surface sprayed in the orchard*

Lead arsenate	Concentration	Spreader used	Mgm. per 100 square inches
No. 1.....	2 to 100.....	None.....	26.4
Do.....	2 to 100.....	Casein 0.05 per cent.....	31.6
Do.....	2 to 100.....	Skim milk 0.60 per cent.....	36.2
Do.....	2 to 100.....	Casein 0.05 per cent.....	25.2
No. 2.....	2 to 100.....	None.....	24.3
Do.....	2 to 100.....	Casein 0.05 per cent.....	29.3
No. 3.....	4 to 100.....	None.....	45.2
Do.....	4 to 100.....	Casein 0.05 per cent.....	55.5

From the results in Table V it will be noted that much more lead arsenate adhered to the leaves sprayed under orchard conditions than when sprayed in the laboratory. This may be due to the carbon dioxide of the breath used to force the spray on the leaves and to the force used in applying the spray. The amount of lead arsenate that adhered was generally higher on the leaves where a spreader had been used than where lead arsenate only had been applied. Skim milk, apparently, was as good as the casein-lime spreaders. It spread well and formed a good even coating of the poison on the leaf surface.

Both the laboratory and orchard experiments indicate that skim milk or casein-lime spreader may be used advantageously if properly applied. The amount of spreader to use for best results is an important phase of the work and must be decided by field observations. Many variable factors that exist at the time of application should be taken into consideration and the amount of spreader used should be controlled accordingly. The pressure or force used, the kind of surface to be covered, the type of gun or nozzle used, and the climatic conditions, may influence the amount of spreader that will give best results. Too much spreader may cause excessive drip if sprayed to the drenching point. On the other hand, drenching with good force with a minimum amount of spreader is advisable. Most of these points should be definitely settled by field observations.

SUMMARY

No definite proportional relationship could be established between the surface tension values of spreader solutions and the observed spreading properties of the different substances. However, a solution having a low surface tension or a low interfacial tension to oil probably has spreading properties.

Of the materials tried, water-soluble protein substances gave best spreading at lowest concentration for the greatest number of surfaces tested. Skim milk, neutralized with hydrated lime, and certain other milk products appear to be the best material for practical purposes.

The concentration of a spreader solution that will give best results depends upon a number of variable factors, such as the type and age of the surface to be sprayed, the force used, and climatic conditions.

The amount of lead arsenate that adheres to the leaf surface is approximately the same when a spreader is added as when one is not.

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INVESTIGATIONS

ASSOCIATIONS BETWEEN NUMBER OF KERNEL ROWS, PRODUCTIVENESS, AND DELETERIOUS CHARACTERS IN CORN¹

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INTRODUCTION

Two different methods of selecting seed corn have been used for many years in the southern part of the United States. Under one of them, seed has been selected from plants bearing two or more ears; under the other, seed has been selected on the basis of the size of the ear. As a result, two classes of varieties, prolific and nonprolific, are now in common use. In general, the prolific varieties bear more than one ear on a plant, whereas the nonprolific varieties bear only one. The ears in the prolific varieties are smaller, and it would seem that the yields of the two might be equal, the differences in size and in number of ears offsetting each other. Experiments with comparable varieties have shown otherwise. Studies of the ears in prolific and nonprolific varieties showed that there were characteristic differences between them in the number of rows of kernels on the ear and in the size and angularity of the kernels.

The effect of differences in these characters upon yield has been investigated, using groups of ears within both prolific and nonprolific varieties and within a number of F_1 varietal crosses. The relation of the number of rows of kernels to smut resistance, to freedom from abnormalities, and to general vigor and productiveness also has been studied in self-fertilized lines. It is the object of this paper to present the data from these experiments and to discuss their application to corn improvement.

EXPERIMENTAL METHODS

The soil and climatic conditions where the experiments were made usually gave yields of 30 to 50 bushels of corn per acre with a good local variety. The different varieties or selections compared required practically the same time to mature, which was well within the seasonal limits.

From 20 to 50 ears were used to represent each variety or selection. Seed ears were selected in the studies on number of rows, etc., that differed in the character being studied but that were as nearly alike in other respects as the material permitted. All of the seed used was examined closely, and defective or irregular kernels were eliminated before planting.

Specially constructed hand planters were used with which an exact number of kernels could be planted at a given place and covered uniformly. The hills were spaced 3 feet apart in the row, center to center, by means of a metal spacing cable. The number of plants per acre was adjusted in accordance with soil productiveness by regulating the space between the rows.

¹ Received for publication Sept. 17, 1924; issued September, 1925.

The hill-checking method was used in all comparisons—that is, one test plant and one check plant were grown in each hill. These two plants were 8 to 10 inches apart in the rows, and always in the same order so that they could be identified. The seed for the test plants in any one row was of one of the varieties or selections being compared. The seed for the check plants was composited by taking a definite number of kernels from a definite number of ears and was the same for all of the rows in the experiment.

In general, two kernels were planted where one plant was desired, the extra seedlings being removed when they were from 4 to 8 inches high. This partly eliminated irregularity in stand. The effect of the remaining irregularity in stand, as well as the effect of soil variation, was avoided by harvesting only perfect hills. A perfect hill was defined as one containing a check plant and a test plant that could be identified definitely and that apparently had had equal growing conditions.

Each variety or selection in an experiment was grown in single rows replicated in different parts of the plat. The perfect hills in these rows were harvested, and the production of the test plants and of the check plants was determined for each row. The weight of ears from the test plants in all rows of one variety or strain in the experiment, divided by the weight of ears from the corresponding check plants, expressed as a percentage, constitutes a relative yield as here used. It is believed that the reliability of results obtained by the hill-checking method is proportional in general to the total number of perfect hills grown. Accordingly, the number of perfect hills on which the relative yields are based is given in the data.

In the varietal experiment in 1912 moisture determinations were made on the shelled grain, and these are given in the data, but no corrections were made in the yields. In most of the experiments the ears were uniformly dry at harvest, or, if not, corrections were made on the basis of data from drying samples.

VARIETAL EXPERIMENTS

EXPERIMENTS IN SOUTH CAROLINA IN 1912

The varietal experiments in South Carolina in 1912 were conducted on 10 farms in different parts of the State. Ten varieties, each of which had been grown previously on one of these farms for three or four years, were used throughout the experiments. One hill-checked row of each of the 10 varieties constituted a section which was replicated 10 times, each of the varieties being used as the check in one section.

The total yield of all the plants of a variety in the experiment was divided by the total yield of all the plants grown in the same perfect hills with it to obtain its relative yield. Computed in this way, the relative yields are percentages of the average yield of all the varieties in the experiment.

The experiment was destroyed by storm and flood on one farm. Two varieties were found to be somewhat earlier than the others, and their yields are not presented. This leaves the yields obtained on 9 farms from 8 varieties, 4 prolific and 4 nonprolific, as a basis for comparison. It was evident that these widely distributed farms repre-

sented different growing conditions, so that the combined data from them may be looked upon as representing the average reaction of these varieties to nine different cropping seasons.

A summary of the data from these experiments is shown in Table I, those from the prolific and nonprolific varieties being grouped separately. The prolific varieties averaged more than one ear per plant, and the nonprolific averaged less than one ear. It is evident from the data that the nonprolific varieties must have had a considerable proportion of barren plants. The average relative yield of the prolific varieties was 9 per cent greater than that of the nonprolific, notwithstanding the fact that the ears contained less moisture² at the time the weights were taken. The most prolific variety in the experiment also was the most productive, and all of the prolific varieties were more productive than any one of the nonprolific except variety No. 5.

TABLE I.—Relative percentage yields of prolific and nonprolific varieties on nine farms in different parts of South Carolina in 1912

Prolific varieties					Nonprolific varieties				
Variety No.	Perfect hills	Relative yield	Moisture	Ears per plant	Variety No.	Perfect hills	Relative yield	Moisture	Ears per plant
		<i>Per cent</i>	<i>Per cent</i>				<i>Per cent</i>	<i>Per cent</i>	
7	5,933	119	16.20	1.45	5	5,963	115	17.65	0.95
4	5,714	113	16.29	1.29	2	5,528	100	17.97	.92
3	5,938	109	16.04	1.12	1	5,818	93	17.03	.97
8	5,941	106	15.81	1.28	10	6,052	88	17.70	.85
Total..	23,526	112	-----	1.28	-----	23,361	103	-----	.92

* Computed directly from the basic data.

EXPERIMENTS IN GEORGIA IN 1915

Varietal experiments were conducted on each of four farms near Thomasville, Ga., in 1915. The growing conditions on each farm were different, and the results may be considered as the average reaction from four cropping seasons.

In planning these experiments it was the intention to include all of the varieties with outstanding characteristics that are commonly grown under climatic conditions similar to those under which the experiments were conducted. Twelve of the thirteen varieties included were studied carefully on the farms from which they were obtained in the fall of 1914, and the seed ears for planting were characteristic of the varieties. A summary of the data from these experiments is shown in Table II, the prolific and nonprolific varieties being grouped separately.

As a class, the prolific varieties had larger yields, more ears per plant, smaller percentages of barren plants, smaller ears per productive plant, and fewer rows of kernels per ear, than did the nonprolific varieties.

² The moisture determinations were made from samples of shelled grain taken from each plot. They do not take into consideration the moisture in the cobs. As the yields are based on the weight of ear corn, no attempt was made to correct for differences in moisture.

TABLE II.—Relative percentage yields of prolific and nonprolific varieties on four farms near Thomasville, Ga., in 1915

Variety	Perfect hills	Relative yield	Ears per plant	Length of total ears per bearing plant (A)	Average diameter of ears (B)	Yield index (A × B ²)	Barren plants	Kernel rows per ear
Prolific varieties:		<i>Per cent</i>		<i>Inches</i>	<i>Inches</i>		<i>Per cent</i>	
Garrick.....	495	146	1.32	7.9	1.9	28.5	4.8	11.61
Wannamaker.....	476	142	1.20	7.9	1.9	28.5	5.5	12.41
Weekley.....	482	130	1.25	7.2	1.8	23.3	6.2	12.84
Whaley.....	408	126	1.29	7.4	1.8	24.0	6.1	12.64
Whatley.....	467	124	1.59	6.3	1.8	20.4	5.1	13.38
Round.....	472	103	1.34	7.3	1.6	18.7	8.9	11.44
Littlecob.....	308	86	1.29	7.8	1.5	17.6	7.1	10.18
Total or average.....	3, 108	124	1.33	7.4	1.8	23.0	6.2	12.07
Nonprolific varieties:								
Loveless.....	462	110	.86	8.4	1.9	30.3	9.7	13.62
Watson.....	448	107	.98	7.8	1.9	28.2	14.1	13.60
Stone.....	327	98	.88	8.0	1.9	28.9	14.7	13.96
Cochran.....	464	87	.91	7.5	1.8	24.3	16.8	13.68
Gwaltney.....	395	87	.90	7.2	2.0	28.8	14.7	12.64
Laguna.....	390	69	.91	7.3	2.1	32.2	15.5	14.69
Total or average.....	2, 486	93	.91	7.7	1.9	28.8	14.1	13.70

* Computed directly from basic data.

The data in column 7 of Table II were obtained by multiplying the average length of total ears per bearing plant by the square of the average diameter of the ears. This gives an index of the volume of ear produced per bearing plant and consequently an index to the yield of the bearing plants. On the basis of the yield index the prolific varieties made their higher yield with a production per bearing plant that actually was less than that of the nonprolific varieties.

Assuming that selection aims to produce the largest individual plant yields, the breeders of the nonprolific varieties had evidently done more effective work than the breeders of the prolific varieties, if only the bearing plants are considered. For some reason, however, the returns were smaller. This was due to the fact that the nonprolific varieties had a disproportionate number of plants without grain. Each of the nonprolific varieties had a larger percentage of barren plants than any one of the prolific varieties, the average percentage of barren plants being 14.1 per cent for the nonprolific group and 6.2 per cent for the prolific group.

COMPARISONS WITHIN VARIETIES

In general, the ears in the nonprolific varieties in the preceding experiments had more kernel rows and more angular, tighter-fitting kernels than the ears in the prolific varieties. In order to study the relation of such differences to yield, a series of experiments was begun in 1914. In all of these experiments, groups of ears were selected that differed in one or more characters of the ears or kernels. The relative productiveness of these groups was then determined.

Many experiments have been conducted on the relation between yield and the different characters of seed ears. The results of these were summarized by Richey³ in 1922 as follows:

³ RICHEY, F. D. THE EXPERIMENTAL BASIS FOR THE PRESENT STATUS OF CORN BREEDING. Jour. Amer. Soc. Agron. 14:4. 1922.

Inasmuch as these data are based on comparisons between ears all of which were suitable for seed, the preponderance of evidence in certain cases seems convincing in spite of the fact that the determining differences in yield are small. There is every indication that selection on the basis of production (weight of ear in this case) is of value. Likewise it is indicated that it is preferable to obtain production by adding to the length rather than to the circumference of the ears, and that smoother, fewer-rowed ears with a lower shelling percentage than the standard show type are inclined to be the better yielders.

Kiesselbach ⁴ stated in substance that in a six-year comparison the long, slender, smooth ears surpassed all other kinds, including the long, large, rough ears; the short, large, rough ears; the short, slender, smooth ears; and unclassified seed of Nebraska White Prize, the variety from which the selections were made. He does not state but shows in an illustration that the most productive type had markedly fewer rows than the ordinary Nebraska White Prize. He also shows in the same bulletin that hybrids producing smooth, few-rowed ears yielded more than hybrids producing ears with a larger number of rows and rougher kernels.

Several experiments were conducted at different points in South Carolina and Florida. The performances of groups of ears differing in the number of rows of kernels were compared at Lykesland, S. C., in 1914, 1916, and 1917; at Brooksville, Fla., in 1917; and at Darlington, S. C., in 1915 and 1916. In the experiments at Lykesland the ears with different numbers of rows were classified further on the basis of angularity of kernel in 1914 and on the basis of kernel size in 1916. Additional studies of the relation of angularity of kernel to yield were made within a variety at Lykesland in 1914 and 1915, and at Darlington in 1915, and within two series of varietal crosses at Darlington in 1914. Summaries of the data from these experiments are given in Tables III, IV, V, VI, and VII.

NUMBER OF KERNEL ROWS AND YIELD

Data on the relation of number of kernel rows to yield in the Roger and Williamson varieties are shown in Table III. These are typical nonprolific varieties, the ears being rough, with comparatively many kernel rows and tightly spaced angular kernels. Three groups of ears in each variety were compared in 1915, and six groups in the Roger variety were compared in 1916.

TABLE III.—*Data on the relation of the number of kernel rows to yield in the Roger and Williamson varieties at Darlington, S. C., in 1915 and 1916*

Points considered	1915						1916					
	Williamson			Roger			Roger					
Number of rows on seed ears.....	12 and 14	16 and 18	20	12	14 and 16	18	10	12	14	16	18	20
Number of perfect hills grown.....	602	506	530	462	414	453	730	751	763	778	727	700
Relative yields.....	108	107	97	106	94	88	98	100	105	96	94	95

The yields were inversely proportional to the number of kernel rows in both varieties in 1915. The highest yield in 1916 was from

⁴ KIESELBACH, T. A. CORN INVESTIGATIONS Nebr. Agr. Exp. Sta. Research Bul. 20, 151 p. 1922.

the ears with 14 rows, the modal number in the Roger variety. Each of the three few-rowed groups (10, 12, and 14 rows), however, yielded more than any one of the three many-rowed groups.

The data show a consistent relation between the number of rows on the seed ears and the yield produced from them, the ears with the lower number of rows yielding more as a class than those with the higher number of rows.

The Garrick variety was included in both the South Carolina and Georgia varietal experiments and was highest yielding in each. From the standpoint of selection, this variety is the most perfect product of the prolific ideal that was studied. It was learned by interview that Mr. Garrick had grown this corn at Weston, S. C., for about 30 years. His ideal had been the production of two or more ears per plant, and he had given no special attention to the character of the ears and kernels. Several thousand ears of this variety were studied on the home farm. The ears were large for the prolific class of corn, the kernels were intermediate between angular and rounded, and the characteristic numbers of rows of kernels on an ear were 10 and 12. The indentation was distinct and smooth to slightly rough.

Data on the relation of number of rows of kernels to yield in the Garrick variety are shown in Table IV. As different lots of ears were used in each experiment, eight distinct comparisons are shown. Ears with the fewest rows of kernels gave the highest yields in six comparisons, and ears with the most rows gave the lowest yields in seven comparisons. Ears with 12 rows of kernels yielded more than those with more than 12 rows, in all of the eight comparisons.

TABLE IV.—Data on the relation of number of kernel rows, angularity of kernels, and weight of kernels in the parent ears to yield in the Garrick variety grown at different places and in different seasons

Place and date of experiment, and character of kernel classes compared in 1914 and 1916	Relative yields from ears with stated number of kernel rows (per cent)						Perfect hills grown from ears with stated number of kernel rows						Weight of kernels (grams) per 1,000 from ears with the stated number of kernel rows			
	8	10	12	14	16	Average ^a	8	10	12	14	16	Total	10	12	14	Av.
Lykesland, S. C., 1914:																
Kernels angular.....		91	84	83		87		317	291	295		903				
Kernels intermediate.....		99	101	96		99		319	295	292		906				
Kernels rounded.....		89	93	84		88		264	300	315		879				
Total or average ^a		94	93	87				900	886	902						
Lykesland, S. C., 1916:																
Kernels large.....		119	100	89		102		606	619	657		1,882	466	418	374	419
Kernels midsized.....		101	92	86		93		644	685	595		1,924	387	378	346	370
Kernels small.....		94	93	86		91		715	602	671		1,988	355	334	324	338
Total or average ^a		104	95	87				1,965	1,906	1,923			403	377	348	
Lykesland, S. C., 1917.....	112		109		100		1,085		579		572					
Brooksville, Fla., 1917.....	104	101	100	94	95		650	630	1,220	1,144	599					

^a Computed directly from the basic data.

Both in the prolific and the nonprolific varieties that were studied there was a strong general tendency for the ears with fewer rows of kernels to yield more than those with larger numbers of rows.

ANGULARITY OF KERNEL AND YIELD

One characteristic difference between the prolific and nonprolific varieties was in the shape of the kernels. The nonprolific varieties tended to have more angular kernels that are crowded more closely on the ear. This angularity probably is due, in part at least, to the greater lateral pressure during their development.

Data on the relation of angularity of kernel to yield in one experiment with the Garrick variety, in which the ears with rounded kernels yielded 9 per cent more than those with angular kernels, are shown in Table V.

TABLE V.—*Data on the relation of angularity of kernels on the seed ears to the number of kernel rows and to yield in the Garrick variety at Lykesland, S. C., in 1914*

Angularity of kernels on seed ears	Kernel rows on seed ears (average)	Perfect hills	Relative yields
Angular	13.2	1,542	<i>Per cent</i> 93
Rounded ^a	10.8	1,735	102

^a This class included kernels of both the intermediate and rounded classes of other experiments.

In 1913, one row of plants of each of 15 varieties was grown in a field of the Roger variety and detasseled before pollen was shed. A similar series was grown in a field of Garrick. The ears of the resulting crosses were grouped on the basis of angularity of kernel and these groups were compared, with the results shown in Table VI.

TABLE VI.—*Data on the relation of angularity of the kernels on the parent ears to yield in two series of varietal crosses at Darlington, S. C., in 1914*

Shape of kernels	15 varieties crossed by Roger			15 varieties crossed by Garrick		
	Rows on parent ears (average)	Perfect hills	Relative yields	Rows on parent ears (average)	Perfect hills	Relative yields
			<i>Per cent</i>			<i>Per cent</i>
Angular	16.1	1,882	105	14.8	1,919	119
Intermediate	12.9	1,848	110	12.9	1,934	123
Rounded	13.7	1,885	108	13.7	1,898	125

The number of plants of each female parent variety was so limited that the rounded-kernel class was poorly represented in some crosses.

The ears with angular kernels produced the lowest yields in both series of crosses. The ears with kernels of intermediate angularity produced the highest yields in the Roger crosses, whereas the ears with kernels of intermediate angularity produced more than the ears with angular and less than those with rounded kernels in the Garrick crosses.

In the preceding experiments the ears with angular kernels had more rows of kernels than those with rounded kernels. To avoid any possible effect of the number of kernel rows on yield, the classi-

fication for angularity also was made within groups of ears of the Garrick variety having the same number of rows of kernels. Data from these experiments are given in Tables IV and VII.

As the data in Table IV are for different lots of ears and those in Table VII are for the same lots of ears grown in different places, four distinct comparisons are provided in the two tables. The ears with kernels of intermediate angularity produced more than either of the extreme classes in all four comparisons. In two of the comparisons the ears with rounded kernels produced significantly more than those with angular kernels and in the other two comparisons the differences were negligible.

SIZE OF KERNEL AND YIELD

In connection with the experiments at Lykesland in 1916, the ears which had been classified for number of rows of kernels were grouped further into those with large, midsized, and small kernels. The data from this experiment are shown in Table IV.

TABLE VII.—Data on the relation of weight and angularity of kernels on the parent ears to the yield of 12-rowed ears of the Garrick variety grown at Darlington and Lykesland, S. C., in 1915

Shape of kernels	Weight per 1,000 kernels	Grown at Darling- ton, S. C.		Grown at Lykes- land, S. C.	
		Perfect hills	Relative yields	Perfect hills	Relative yields
	Gm.		Per cent		Per cent
Angular.....	382	958	88	765	88
Midangular.....	399	905	99	798	102
Midrounded.....	397	3, 852	102	3, 330	102
Rounded.....	379	3, 778	96	3, 332	97

The yields tended to be directly proportional to the size of the kernels, and the yield of the ears having both the fewest rows and the largest kernels was very distinctly the highest. It will be noted in Table VII that the ears with midangular and midrounded kernels had heavier kernels than those of the extreme classes. The larger yields of these intermediate groups, therefore, may have been a result of their having heavier kernels, although this is not certain.

INTERRELATION OF NUMBER OF KERNEL ROWS, ANGULARITY OF KERNEL, AND SIZE OF KERNEL

It has been shown in connection with some of the preceding experiments that angularity of kernel is related to weight of kernel and that both of these characteristics are related to the number of rows of kernels on an ear. These interrelations probably are universal within a variety, and it is not possible to separate the effect of each individual factor in these experiments. Fortunately, however, this is not necessary from the present point of view, as it is necessary to know only that ears chosen within a variety to represent similar combinations of these characters react in a similar manner. Both in the varietal experiments and in the comparisons within varieties, the groups of ears with fewer rows of kernels and with less angular kernels have produced higher yields than those with more rows and more angular kernels

EXPERIMENTS WITH SELF-FERTILIZED LINES

Systematic selection within self-fertilized lines in several varieties has given comparatively quick results in the isolation of strains with distinct characteristics. In addition, it has afforded a view of some indirect effects of selection that could not be seen in the studies with open-fertilized material. Some of the results are given in the following pages.

EFFECT OF SELECTION UPON THE NUMBER OF ROWS OF KERNELS

Many plants of the Garrick variety were selfed in 1916 and good seed ears were selected from these to represent six classes with different numbers of kernel rows on an ear. These ranged from 8 to 18 rows, inclusive. These classes were maintained in the succeeding years by continuous selection. The effect of the selection during the first five years upon the percentage of progeny ears having the same number of kernel rows as the parent ear is shown in Table VIII.

TABLE VIII.—Data on the effect of selecting continuously within selfed lines for different numbers of kernel rows, upon the percentages of the progeny conforming to the parental number of kernel rows

Kind of data	Kernel rows on parent ears	Years in which the progeny were grown				
		1917	1918	1919	1920	1921
Number of parent ears.....	8	18	8	15	52	49
	10	24	8	19	64	69
	12	24	5	11	53	76
	14	21	4	10	52	76
	16	18	4	8	37	40
	18	3	4	2	15	16
Number of progeny ears classified.....	8	3, 265	1, 076	2, 653	2, 563	2, 499
	10	4, 790	1, 050	3, 486	3, 513	4, 642
	12	4, 903	597	1, 750	2, 352	3, 804
	14	3, 095	498	1, 198	2, 147	3, 432
	16	3, 410	441	835	1, 332	1, 516
	18	572	477	197	530	612
Percentages of progeny ears having the same number of kernel rows as the parent ear.		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
	8	21.2	25.4	47.7	68.6	85.6
	10	45.9	44.2	55.9	63.1	66.5
	12	38.7	38.6	44.9	46.8	52.9
	14	19.1	30.6	33.6	36.3	50.3
	16	11.8	13.0	26.1	32.9	43.9
Highest percentage of progeny ears in an individual ear row having the same number of kernel rows as the parent ear.	18	8.6	4.2	19.7	22.5	43.8
	8	35.9	32.3	86.6	100.0	100.0
	10	66.2	56.8	75.7	93.3	95.0
	12	51.0	49.0	66.9	81.3	77.0
	14	46.2	35.0	45.8	64.3	88.0
	16	26.3	20.6	33.9	59.3	63.0
	18	25.8	11.3	30.9	46.2	60.0

With the exception of the 10, 12, and 18 rowed classes in 1918, there was an annual increase throughout the five years in the degree to which the progeny ears in each class conformed to the parental character. The rate of increase, however, was not the same for each class. In the parent variety the mode for number of kernel rows was 12. During the first three selfed generations the 10-rowed class led in percentage of conformity to the parental number and the 8-rowed group took and maintained the lead after that. In the data for 1920 and 1921 the percentage of conformity to the parental number tended to be inversely proportional to the number of rows on the ears.

INDIRECT EFFECT OF SELECTION ON INDENTATION AND ANGULARITY OF KERNELS

After the Garrick lines had been selfed and selected for different numbers of kernel rows for five years they were examined to determine whether there had been any indirect effect upon the indentation and shape of the kernels. The results of this examination are shown in Table IX, the classifications for indentation and angularity being based upon the characteristic tendency of the ears in the individual ear rows. These studies were made at the Arlington Experiment Farm, Rosslyn, Va., and in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station, Florence, S. C.

TABLE IX.—Data on the effect of selecting for different numbers of kernel rows upon the indentation and angularity of kernels of self-fertilized lines of Garrick grown at Florence, S. C.,^a and Rosslyn, Va., in 1921

Kernel rows on parent ears	Ear rows grown	Percentages of ear rows producing ears with mode for kernel indentation stated					Percentages of ear rows producing ears with mode for angularity of kernels stated		
		None	Trace on only a few kernels	Shallow and smooth	Mid-depth and wrinkled	Deep and rough	Round-ed	Inter-mediate	Angular
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
8	46	26.1	6.5	45.7	19.6	2.2	69.6	28.3	2.1
10	59	16.9	6.8	52.5	13.6	10.2	59.3	32.2	8.5
12	59	18.6	8.5	42.4	16.9	13.6	55.9	33.9	10.2
14	65	7.7	1.5	50.8	20.0	20.0	46.2	40.0	13.8
16	52	5.8	3.8	38.5	26.9	25.0	42.3	28.8	28.8
18	34	8.8	0.0	35.3	20.6	35.3	44.1	20.6	35.3

^a The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

The classification for indentation was more or less difficult, but it is believed that it was fairly accurate in the extreme classes. The largest percentages of ear rows with no indentation were from the parents with 8, 10, and 12 kernel rows, and the largest percentages in the class with deep and rough indentation were from parents with 14, 16, and 18 kernel rows. It is evident from this that selection for low numbers of rows on the ears tended to result in a different indentation from that obtained by selecting for higher numbers of kernel rows. Similarly, rounded kernels naturally followed selection for low numbers of kernel rows, and angular kernels followed selection for higher numbers of rows.

RELATION OF SIZE OF EARS TO NUMBER OF ROWS OF KERNELS

All of the ears produced in the Garrick selfed lines in 1921 were measured as to length and diameter. The total length of all the ears from each ear row was divided by the number of bearing plants in that row to obtain the total length of ear per plant. A yield index then was computed by multiplying this value by the square of the average diameter of the ears in the row. These data, and the average number of ears per plant produced by parent ears with different numbers of kernel rows, are shown in Table X.

TABLE X.—*Effect of selecting for numbers of rows of kernels upon number of ears per bearing plant, and length and diameter of ears, in selfed lines of Garrick grown at Florence, S. C.,^a and Rosslyn, Va., in 1921*

Kernel rows on parent ears	Total progeny studied		Ears per plant	Average length of total ears per bearing plant (A)	Average diameter of ears (B)	Yield index (A × B ²)
	Number of plants	Number of ears				
8	1,091	2,366	2.17	<i>Inches</i> 13.21	<i>Inches</i> 1.48	<i>Inches</i> 28.94
10	1,742	3,493	2.01	12.86	1.58	32.10
12	1,587	2,944	1.86	12.67	1.68	35.76
14	1,757	3,209	1.83	12.13	1.75	37.15
16	1,348	2,091	1.55	9.92	1.81	32.50
18	867	1,335	1.54	9.76	1.85	33.40

^a The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

The number of ears per plant tended to be inversely proportional to the number of rows on the parent ear and directly proportional to the length of ear per plant. The length of total ears per bearing plant tended to be inversely proportional, and the average diameter of ear tended to be directly proportional to the number of rows on the parent ears. The yield indexes were largest in the two middle classes for number of rows of kernels. The classes with 16 and 18 rows have larger yield indexes than the classes with only 8 or 10 rows. The yield indexes are based only on bearing plants and the differences are small. Inasmuch as the percentage of barren plants (Table XI) was materially larger in the classes with more kernel rows, the acre yield of the fewer-rowed ears evidently was larger. This agrees, in general, with the data previously presented from open-fertilized corn.

RELATION OF DELETERIOUS CHARACTERS TO NUMBER OF ROWS OF KERNELS

Many abnormalities occur among corn plants in lines that have been self-fertilized in successive generations. Some of these are eliminated by selection and others become more or less fixed and characteristic of individual hereditary lines. All of these abnormal characters have a more or less deleterious effect upon yield.

After five generations of selfing and selection for different numbers of kernel rows, data were obtained on the proportion of plants in the different Garrick lines having certain deleterious characters. The characters considered were: Plaited and erect leaves, entangled leaves, chlorophyl blotch, dead blotch, red and yellow flame, and barrenness. The dead blotch and the red and yellow flame characters both result in the premature death of the plants having them.

The data were obtained on a total of 4,013 plants in 158 ear rows grown in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station, at Florence, S. C., and on 3,167 plants in 105 ear rows grown at the Arlington Experiment Farm, Rosslyn, Va. Notes were taken on all of the deleterious characters in the experiment in South Carolina, but in the experiment in Virginia barrenness and red and yellow flame were the only ones noted. A summary of the data from these experiments, in so far as they relate to the present problem, is shown in Table XI, the data being grouped according to the number of rows on the parent ears.

In both experiments the lines from parent ears with 8, 10, and 12 rows had smaller percentages of plants affected than the lines from parent ears with 16, 18, and 20 rows. This was true for each of the deleterious characters considered, although the differences in plants affected with dead blotch and chlorophyl blotch were small.

TABLE XI.—*Data on the relation of number of kernel rows on parent ears to percentages of plants having deleterious characters in selfed lines of Garrick grown at Rosslyn, Va., and Florence, S. C.,^a in 1921*

Deleterious characters	Percentage of plants affected ^b		Deleterious characters	Percentage of plants affected ^b	
	Parent ears having 8, 10, and 12 rows	Parent ears having 16, 18, and 20 rows		Parent ears having 8, 10, and 12 rows	Parent ears having 16, 18, and 20 rows
Florence, S. C.:	<i>Per cent</i>	<i>Per cent</i>	Florence, S. C.—Continued.	<i>Per cent</i>	<i>Per cent</i>
Plaited and erect leaves.....	39.99	54.46	Barrenness.....	1.61	6.77
Entangled leaves.....	8.03	15.68	Rosslyn, Va.:		
Chlorophyl blotch.....	13.17	13.37	Red and yellow flame....	1.89	7.40
Dead blotch.....	29.45	32.34	Barrenness.....	1.94	8.70
Red and yellow flame.....	.96	16.66			

^a The data at Florence, S. C., were obtained in cooperation with the Pee Dee Substation of the South Carolina Agricultural Experiment Station.

^b The total numbers of plants studied were 4,013 in South Carolina and 3,167 in Virginia.

RELATION OF SMUT RESISTANCE TO NUMBER OF ROWS OF KERNELS

The selfed lines in the Garrick variety were studied in 1922 ⁵ to learn whether there was any evidence of resistance to smut. The studies were made at the Arlington Experiment Farm, Rosslyn, Va., on reclaimed land along the Potomac River where smut infection normally is high. The 138 ear rows that were studied represented 13 of the original selfed lines started in 1917 which had survived six generations of selfing and selection. Some of the ear rows in 5 of the 13 original lines or families were entirely free from smut. Three of these five families had been selected during the first five generations of selfing for 12 or less rows on the ear. The percentages of ear rows free from smut in these families were 33.3, 36.4, and 37.5. Two of the five families had been selected for 14 or more rows during the same period. The percentages of ear rows free from smut in these families were 5 and 25. The average percentage of smut-free ear rows in all of the lines from few-rowed parent ears was 12.2, and in all of the lines from many-rowed parent ears was 4.7.

Studies of smut resistance were continued with the Garrick lines in 1923 and additional investigations were begun with Cuban Yellow, a flint variety from southern Florida, and with Boone County White. Inbred lines of Cuban Yellow that had been selfed for four generations and lines of Boone County White that had been selfed three generations were used. In the selection of the lines in these varieties no attention had been given to the number of kernel rows on the ear. The experiments were conducted at the Arlington Experiment Farm, Rosslyn, Va.

⁵ Notes on infection by smut and the artificial inoculations (referred to later) were made by W. H. Tisdale, Pathologist in Charge of Smut Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and his associates

In order to provide more opportunity for infection, stable manure with which large quantities of smut spores had been mixed was spread on the experimental plat in the spring. Later, a water suspension of conidia of the smut fungus from pure culture was applied to the growing points of the plants after they had been wounded with a sharp wire.

A summary of the data on freedom from smut in the Garrick lines in 1923 is given in Table XII, classified according to the number of kernel rows on the parent ears. Data on freedom from smut in the parent ear rows in 1922 also are given. All of the few-rowed parent ears used in this experiment were from ear rows that were entirely free from smut in 1922, as was one of the many-rowed parent ears. The other seven parent ears of the many-rowed class were from the most nearly smut-free ear rows of this class from which selfed ears were obtained in 1922. Inasmuch as approximately equal numbers of hand pollinations were attempted in the rows of both classes in 1922, this failure to obtain selfed ears from the many-rowed class furnishes an interesting side light on the greater vigor of the plants from the few-rowed parent ears.

TABLE XII.—Data on the relation of number of kernel rows on the parent ears to freedom from smut in selfed lines of Garrick grown at Arlington Experiment Farm, Rosslyn, Va., in 1922 and 1923

Family No.	Parent ears with 8 to 12 kernel rows						Family No.	Parent ears with 14 to 20 kernel rows																				
	Parent rows, 1922			Progeny rows, 1923				Parent rows, 1922			Progeny rows, 1923																	
	Ear row No.	Total plants	Smut-free plants	Ear row No.	Total plants	Smut-free plants		Ear row No.	Total plants	Smut-free plants	Ear row No.	Total plants	Smut-free plants															
49	{	76	20	<i>P. ct.</i> 100.0	{	2	89	49.4	86	{	45	20	<i>P. ct.</i> 95.0	20	117	<i>P. ct.</i> 29.9												
						4	85	51.8									49	22	100.0	22	77	64.9						
						5	103	79.6															69	26	88.5	23	84	31.0
						7	101	75.2																				
54	{	26	17	100.0	{	8	102	80.4	99	{	51	18	94.5	{	25	132												
						10	107	77.6							26	113	61.9											
						11	114	84.2							29	108	61.1											
						13	97	93.8							30	108	29.6											
						14	98	81.6							31	115	17.4											
						16	113	85.0																				
						17	123	90.2																				
						Total ear rows----		76							100.0	-----	1,132	° 78.2	Total ear rows---		124	° 83.9	-----	854	° 44.6			

° Computed directly from the basic data

Only 2 of the 11 rows from ears with from 8 to 12 rows of kernels had lower percentages of smut-free plants than the most nearly smut-free rows from ears with 14 to 20 rows of kernels in 1923, and the rows from ears with fewer kernel rows averaged 33.6 per cent more smut-free plants than the rows from ears with more kernel rows.

The percentages of smut-free plants were lower in 1923 than in the parent-ear rows in 1922. This may have been due to a difference in the seasons, to the artificial inoculation of soil and plants in 1923, or to the fact that the data for 1922 are based only on ear rows as selected having the fewest smutted plants. In any event,

the difference in freedom from smut in the parent-ear rows of the two classes in 1922 was only 16.1 per cent, whereas the difference in 1923 was 33.6 per cent.

The data on freedom from smut in the experiment with the selfed lines of Cuban Yellow and Boone County White varieties are given in Table XIII, arranged according to the number of rows of kernels on the parent ears.

TABLE XIII.—Percentages of smut-free plants in selfed lines of Cuban Yellow and Boone County White from parent ears with different numbers of rows of kernels, at Arlington Experiment Farm, Rosslyn, Va., 1923

Variety	Rows from parent ears											
	12-kernel rows		14-kernel rows		16-kernel rows		18-kernel rows		20-kernel rows		22-kernel rows	
	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants	Total plants	Smut-free plants
Cuban Yellow.	97	<i>P. ct.</i> 73.2	88	<i>P. ct.</i> 51.1	110	<i>P. ct.</i> 31.8	119	<i>P. ct.</i> 47.9		<i>P. ct.</i>		<i>P. ct.</i>
	106	78.3	100	65.0	106	67.0						
	108	60.2	107	58.9								
	92	78.3	112	58.9								
	110	63.6	87	71.3								
	111	59.5	109	55.0								
	118	81.4	108	69.4								
	111	40.5	114	52.6								
	106	50.0										
	106	54.7										
	109	77.1										
Total or average.	1,174	65.0	825	60.1	216	49.1	119	47.9				
Boone County White.	105	37.1	103	90.3	104	31.7	100	38.0	72	72.2	100	54.0
			99	47.5	106	83.0	86	69.8	97	78.4		
			111	91.9	89	47.2	82	72.0				
			95	48.4	91	84.6						
			69	73.9	98	69.4						
			97	83.5	94	51.1						
			105	73.3	99	86.9						
			59	44.1	93	46.2						
			83	67.5	107	89.7						
			65	49.2	112	43.8						
Total or average.	105	37.1	886	69.0	993	63.4	268	58.6	169	75.7	100	54.0

* Computed directly from basic data.

In the Cuban Yellow variety, the ear rows having the largest percentages of smut-free plants were from ears with 12 rows of kernels, and the average percentage of smut-free plants decreased as the number of kernel rows increased.

In Boone County White, the ear rows with the largest percentages of smut-free plants were from ears with 14 rows of kernels. The one ear with 12 rows of kernels produced plants of which only 37.1 per cent was free from smut, and the two ears with 20 rows produced plants of which 75.7 per cent was free from smut. Omitting these classes, there was a consistent negative relation between the percentages of plants free from smut and the number of rows of kernels on the parent ears.

There were but few parent ears with numbers of rows other than those in the two principal classes in the lines of the Cuban Yellow

and the Boone County White varieties. Moreover, these lines had been selfed only for four and three generations, respectively. When the data are considered as a whole, however, they are in agreement with those obtained with the Garrick variety in indicating that lines characterized by relatively low numbers of rows on the ears are more resistant to smut than those characterized by larger numbers of kernel rows.

RELATION OF VIGOR TO NUMBER OF ROWS OF KERNELS

Reference already has been made to the selection of selfed lines of Cuban Yellow and Boone County White and to the fact that no attention was paid to the number of rows of kernels on the seed ears. These lines were selected on the basis of their general vigor, freedom from abnormality, and productiveness.

Most of the ears of the Cuban Yellow had 14 or 16 rows of kernels when selfing was begun. After four generations of selection for vigor, without reference to the number of rows of kernels, nearly all of the breeding ears had 12 or 14 kernel rows, the 12-rowed class being the largest (Table XIII). Most of the ears in the Boone County White variety had 16 or 18 rows of kernels when selfing was begun. After three generations of selection for vigor, without reference to the number of rows of kernels, 20 of the 27 breeding ears had 14 or 16 rows. Thus, selection for vigor and productiveness in the selfed lines of both of these varieties has decreased the characteristic number of rows of kernels on the ear.

The seventh selfed generation of the Garrick lines was grown at Baton Rouge, La., in 1923, in cooperation with the Louisiana Agricultural Experiment Station. Thirteen of the 108 original selfed lines of 1917 were represented in this planting and each of these lines or families was represented by from 3 to 13 ear rows.

The seed ears representing a family in 1923 varied somewhat in regard to the number of rows of kernels in 11 of the 13 families. The ear rows of each of these 11 families were classified into desirable and undesirable rows according as the plants in them approached the normal stability and vigor of open-fertilized corn. This classification was based upon a consideration of the culms, roots, leaves, chlorophyll, ears, and manner and time of dying.

Data on the relation of the number of kernel rows on the parent ears to the desirability or undesirability of the ear rows grown from them are shown by families in Table XIV. The last column in this table shows the differences between the average number of kernel rows on the parent ears producing desirable and undesirable ear rows in each of the 11 families. The parent ears producing desirable ear rows had fewer kernel rows than those producing undesirable ones in 9 of the 11 families, and averaged 1.52 of a row less in all of the rows.

If the future breeding stocks are selected on the basis of the most vigorous and productive ear rows, it is evident that the tendency will be to reduce the number of kernel rows on the ears in the families with higher numbers until the numbers in all families are about the same level. This is in agreement with the change in the number of rows in the Cuban Yellow and Boone County White varieties which showed the effects of this tendency during four years and three years of selection, respectively.

TABLE XIV.—Data on the relation of number of rows of kernels on the parent ear-
to desirability or undesirability of plants in the ear rows from them in 11 selfs
fertilized families of Garrick at Baton Rouge, La., in 1923 ^a

Family No.	Kernel rows on the 1917 selfed seed ears	Classification of the 1923 ear rows	Parent ears with the number of kernel rows stated producing ear rows classed as desirable and undesirable							Total ear rows	Kernel rows on parent ears (average)	Difference between average number of kernel rows on parent ears of desirable and undesirable ear rows
			8	10	12	14	16	18	20			
32	10-----	{Desirable-----	1	1						2	9.00	-0.14
		{Undesirable-----	4	3						7	8.86	
57	10-----	{Desirable-----	6	1	1					8	8.75	3.65
		{Undesirable-----			4	1				5	12.40	
38	10-----	{Desirable-----		2						2	10.00	1.33
		{Undesirable-----				1				3	11.33	
49	12-----	{Desirable-----		1	1					2	11.00	1.67
		{Undesirable-----			2	1				3	12.67	
54	12-----	{Desirable-----		1	4		1			6	12.33	.07
		{Undesirable-----		1	2	2				5	12.40	
79	14-----	{Desirable-----		1		2	3			6	14.33	-1.33
		{Undesirable-----			1	1				2	13.00	
86	14-----	{Desirable-----			1	5		1		7	14.29	.04
		{Undesirable-----			3	1	1		1	6	14.33	
90	16-----	{Desirable-----				2				2	14.00	2.00
		{Undesirable-----				1	1	1		3	16.00	
93	16-----	{Desirable-----					1		1	2	17.00	3.00
		{Undesirable-----							1	1	20.00	
99	16-----	{Desirable-----				2				2	14.00	2.40
		{Undesirable-----			1		1	3		5	16.40	
107	18-----	{Desirable-----			1		1			2	14.00	4.00
		{Undesirable-----					1	1	1	3	18.00	
All families-		{Desirable-----								41	12.61	1.52
		{Undesirable-----								43	14.13	

^aThe data were obtained in cooperation with the Louisiana Agricultural Experiment Station.

DISCUSSION

Corn breeding probably has received more attention in this country than any other problem of crop improvement. If it were dependent only upon the selection of strains with constructive characters, such as size of ear, it would hardly be a serious problem. Yet this vast experience has created among the thoughtful only uncertainty and caution in making general recommendations. The possibilities of selection within self-fertilized lines as a means of corn improvement are being investigated extensively. The problem is not an easy one, however, and it is impossible to predict what the future may bring in the way of a practical application of this principle. In any event, the bulk of the seed corn planted in the United States will be obtained by mass selection for some time to come, and it therefore is important to know what characters, if any, can be used safely as a guide in such selection.

The experiments with open-fertilized seed reported give further evidence that seed ears with larger numbers of kernel rows and smaller and more angular kernels tend to produce lower yields. This was true in some of the experiments in spite of a higher yield per bearing plant because the seed from the many-rowed ears produced more barren plants. The data from the experiments with self-fertilized lines indicate that the larger proportion of weak and barren plants in strains with many-rowed ears was due to a tendency for such strains to have more deleterious characters.

Selection for large ears frequently has resulted in ears with larger numbers of kernel rows, and it seems probable that the benefits accruing from increased size of ear may have been nullified for the reasons already noted. The diameter of ear and the number of rows of kernels are correlated, though how much of this relation is unavoidable is unknown. Size of ear is dependent upon length as well as diameter, however, and the number of kernel rows need not be increased in obtaining longer ears. It therefore seems that selection on the basis of total length of ear (or ears) per plant, together with as much diameter as may be had without too many rows of kernels, may be recommended as satisfying the size requirements without increasing the ill effects that apparently are associated with a larger number of kernel rows. It is probable that the maximum length of total ear per plant can be obtained by having more than one ear on a plant. Whether this is necessary to obtain the maximum yield, however, or whether plants bearing one long ear may be equally productive under some conditions, remains to be determined.

SUMMARY

Experiments are reported in which prolific varieties were more productive as a class than comparable nonprolific varieties. The ears in the prolific varieties had fewer rows of kernels and smaller diameters and the kernels were less angular than in the nonprolific varieties.

In comparisons between groups of ears, both within prolific and nonprolific varieties, and within several F_1 crosses, those with fewer kernel rows, larger kernels, or less angular kernels were more productive. In the comparisons, both between and within varieties, the lots with fewer kernel rows produced fewer barren plants and, in some cases, made the larger total yield in spite of a smaller yield per bearing plant as measured by the yield index.

Selection for different numbers of kernel rows in selfed lines resulted in an essentially steady increase during five generations in the degree of conformity to the parental number. The rate of this increase was greatest in the lines with the fewest rows and decreased as the number of rows increased. Selection also resulted incidentally in other differences. Thus, the lines having smaller numbers of kernel rows had a greater length of ear per plant, ears with a smaller diameter, and kernels more rounded and with less indentation than the lines having larger numbers of kernel rows. Finally, the fewer-rowed lines were more resistant to corn smut, had fewer plants with heritable deleterious characters, and were more vigorous and productive in general.



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BEAN VARIETAL TESTS FOR DISEASE RESISTANCE¹

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INTRODUCTION

The discovery or development of disease-resistant varieties of beans (*Phaseolus vulgaris* L.) has been recognized for several years as the most promising line of investigation looking toward the prevention of large annual losses. Several American and European investigators have published valuable evidence on the reaction of the commoner bean varieties to the different diseases. Unfortunately, most of the standard American varieties have proved to be susceptible to one, two, and frequently all the major troubles.

Hybridization has been resorted to, but this is complicated and time-consuming in securing resistance to anthracnose because of the variation in reaction among the varieties to an undetermined number of biologic forms of the causal fungus, *Colletotrichum lindemuthianum* (Sacc. and Magn., B. and C.). No variety resistant to all the known biologic forms has hitherto been reported.

The success of the Cornell workers in combining the specific disease resistance and other desirable characters of two or more varieties of beans into one strain is sufficiently well known to need no review. Considering the many different types of bean varieties, their geographic distribution, climatic adaptation, and market requirements, the necessity for an abundance of resistant types with which to work is realized. The absence of such has been a serious handicap to the plant breeder. This paper presents the results of an extensive search for resistant material.

A large collection of local and foreign varieties and species of beans has been made and tested. Although it was realized that many of the foreign varieties would probably not be adapted to direct commercial utilization in the United States, they might, through possession of unusual resistance to one or more diseases, be valuable for hybridization with susceptible American types. While the information here given is in many respects preliminary in character, in others it seems conclusive enough to warrant bringing it to the attention of other investigators.

NOMENCLATURE AND AUTHENTICITY OF VARIETIES USED

The names adopted by Jarvis (6)³ have been generally used in listing the standard American varieties. Those introduced since the publication of his bulletin in 1908, or of uncertain classification, are

¹ Received for publication June 30, 1924; issued September, 1925.

² Resigned April 2, 1925.

³ Reference is made by number (*italic*) to "Literature cited," p. 153.

given the names advertised by the seedsmen introducing them. All foreign varieties are listed by the names accompanying the original importation and, excepting the standard European sorts, also the seed and plant introduction number (S. P. I. No.) of the Office of Foreign Seed and Plant Introduction of the Bureau of Plant Industry through which they were obtained. Information on the European varieties can be readily secured by consulting the accredited seedsmen's catalogues for the years during which the tests were made; or, better, standard horticultural treatises, such as Denaiffe (4), and Robinson (10), who give also the synonymy. Some of the varieties here included are no longer listed by seedsmen. All lots were carefully examined for trueness to name and very few showed mixtures or sports.

The foreign varieties described by European horticulturists and given English and American names synonymous with standard American varieties are for the most part identical with the American varieties of the same name, as shown by a comparison of the European description with the descriptions of Tracy (9), Irish (5), and Jarvis (6), as well as from the writers' field observations. Occasionally, however, the descriptions do not agree, and the European and American varieties of the same name are obviously different. Even where they are apparently identical in horticultural characteristics, the reaction to disease may be different, and, for the purposes of this study, they are treated separately.

Where no name accompanied the original importation there is given a short description of the seed, based on samples from the original packet. Since many of the non-European foreign varieties were purchased in native markets, varietal mixtures and hybrids were common. Of special interest in this connection is the large assortment of brilliantly colored and variegated types grown by the natives of East Africa, particularly of Urundi Province. Among the beans from this source were many of obviously hybrid origin, as shown by their segregation into various types following the first planting in this country. Except where the amount of crossing was slight, the tests on the samples containing volunteer hybrids have not been published. The mixtures and hybrids were in all cases separated as far as possible from the "type" seed, but in many instances were not tested until 1923, when a larger field plot was available.

Since the original samples in many cases were small, it was necessary to save seed each year from the test rows in order to continue the work. The small percentage of volunteer hybrids resulting from this practice have been largely eliminated by subsequent selection in cases where the variety proved to be of value. Many of the purely tropical varieties failed to mature seed in the latitude of Lansing, Mich., and were later grown for increase purposes in Florida. To what extent the excessive vegetative growth of such types at Lansing may have influenced their natural resistance or susceptibility to the various diseases is not known.

DISEASES INCLUDED IN RESISTANCE STUDIES

Attention has been directed principally toward the discovery of varieties or strains of varieties possessing resistance to bacterial blight (*Bacterium phaseoli* E. F. S.) and to the various biologic forms of the anthracnose fungus (*Colletotrichum lindemuthianum* (Sacc.

and Magn., B. and C.), which are doubtless the two most important diseases of beans in the eastern United States. Incidental to tests with these diseases, notes were made on the natural occurrence and severity of mosaic. The work in 1923 also included greenhouse and field inoculations with bacterial wilt (*Bacterium flaccumfaciens* Hedges), a disease until recently confused with bacterial blight, and of increasing economic importance in the northern bean sections.

Authentic virile cultures of the bacterial blight and wilt organisms were obtained from Florence Hedges, of the Laboratory of Plant Pathology of the Bureau of Plant Industry, United States Department of Agriculture, and by isolations from typically diseased plants from Michigan and New York. Tested cultures of the *alpha*, *beta*, and *gamma* biologic forms of the anthracnose fungus were obtained from W. H. Burkholder, of Cornell University; and the eight biologic forms (Nos. I to VIII, inclusive), as well as samples of the bean varieties and strains used to differentiate them, were kindly furnished by J. G. Leach, of the University of Minnesota. The *alpha* and *beta* forms only were used in the inoculations of 1920 to 1922, inclusive, while in 1923 there were added the *gamma* form described by Burkholder (2) and also the above-mentioned eight biologic forms recently described by Leach (8). Since some of the varieties listed in Table II were not tested in 1923, they were presumably exposed only to the first two forms, a possibility which should be remembered in judging their behavior. Although some separate greenhouse inoculations are recorded in this paper for certain of the forms, no detailed study to determine resistance or susceptibility to individual biologic forms has been made. The object has been to find, if possible, suitable types immediately available for the plant breeder, which combine resistance or immunity to all the forms. The whole subject of biologic forms in the bean *Colletotrichum* requires further investigation and, at least until the relationships between the 11 forms thus far reported are established, extensive variety testing with individual forms is scarcely advisable.

LOCATION, DURATION OF TESTS, AND METHODS EMPLOYED

FIELD STUDIES

The field investigations here reported extend over the period 1920 to 1923, inclusive. In 1920 a single test plot was located near Saginaw, Mich., in cooperation with the local farm bureau. During the succeeding years the work was centered at East Lansing, Mich., where land, laboratory facilities, and much assistance were furnished by the departments of botany and farm crops of the Michigan Agricultural College.⁴ Since weather conditions frequently prohibit the best development of anthracnose at Lansing, it was necessary, in order to obtain a severe test of the varieties every season, to establish additional plots containing partial replications of the Lansing test in more favorable places. Accordingly in 1921 and 1923 a plot was maintained in the northern peninsula of Michigan near McMillan in cooperation with the Jerome B. Rice Seed Co. In 1923, one was placed in the northern "Thumb" section of Michigan near Bad Axe, in cooperation with the local farm bureau, and another at St. Paul,

⁴ Credit is due G. A. Meckstroth for assistance at Saginaw during 1920, and to E. F. Hopkins, E. V. McKenna, and Zadik Voscan for handling the details of the work at East Lansing in 1923.

Minn. The Minnesota test was conducted by J. G. Leach, who planted the seed, made the inoculations, and kindly furnished the authors with a copy of his notes on disease prevalence.

The field plots were generally laid off in rows, 3 to 4 feet apart and from 100 to 200 feet long, divided into rod units, each unit being planted to a single variety which was sometimes replicated in other parts of the same plot. As a rule every tenth row was planted with infected seed of a very susceptible type, to serve as a control and to supply inoculum to the adjacent lots.

In addition to frequent artificial inoculations, natural infection and spread of the diseases were facilitated by scattering between the rows severely diseased vines collected and preserved from the previous year. The artificial inoculations were made by atomizing the plants from three to five times during the season under moist weather conditions, with combined spore suspensions from pure cultures of the different biologic forms of *Colletotrichum lindemuthianum* and with bacterial suspensions of the blight organism. No separate inoculations with the biologic forms of anthracnose were undertaken in the field.

In an acre portion of the 1923 East Lansing plot the most resistant varieties and selections discovered during the preceding years were segregated and given an unusually severe test. Following the method employed by Burkholder and Emerson of Cornell infected seed of very susceptible varieties were planted in "buffer" rows spaced about 12 inches from the supposedly resistant type to be tested. Abundant infection was further secured in this plot by the aid of oscillating sprinklers and by cheesecloth inoculation cages kept continuously moist by properly directed spray nozzles (pl. 1).

The bacterial-wilt inoculations, limited to preliminary tests in the summer of 1923, were made both on young seedlings and on tender growing parts of older plants. Ten plants of each variety were inoculated when 8 to 12 days old by simply jerking off one or both of the shrunken cotyledons prior to formation of the abscission layer and applying a drop of bacterial suspension from a 3 to 4 day old potato cylinder culture. The figures in the eleventh column of Table II give the results of these tests. The first series of inoculations on older plants was made when the plants were approximately 3 weeks old. The bacteria were pricked into the young growth, stems, petioles, and runners, at 2 or 3 different parts of the plant, with a flamed needle. Since after 10 days but few of the varieties showed evidence of infection, a second attempt was made, in which not only the young growing parts but also the main stems were inoculated. A large number of the varieties still remaining uninfected up to the flowering and podding stage were inoculated a third time in the same way, but again without much success. As shown later the "cotyledon method" was found much more certain of securing constant and uniform infection and was much quicker, but the period in the growth of the plant during which it can be carried out is too short to permit inoculation of a large number of varieties. It should be done only during the stage of growth indicated, since removal of the cotyledons too early is obviously injurious to the plant, and after the abscission layer is formed the vessels in which the bacteria enter are of course closed.



General view of experimental plot at East Lansing, Mich., August 10, 1923, showing arrangement of varieties, cloth inoculation cage, and oscillating sprinklers for producing artificial epidemics of anthracnose and bacterial blight

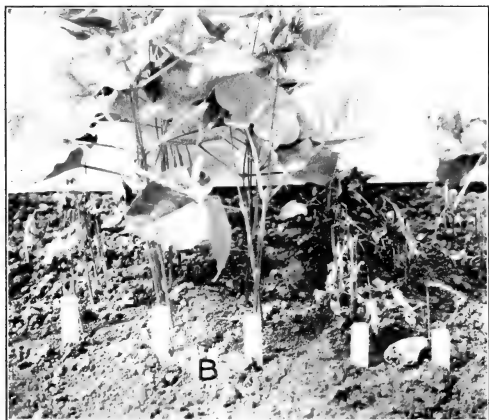
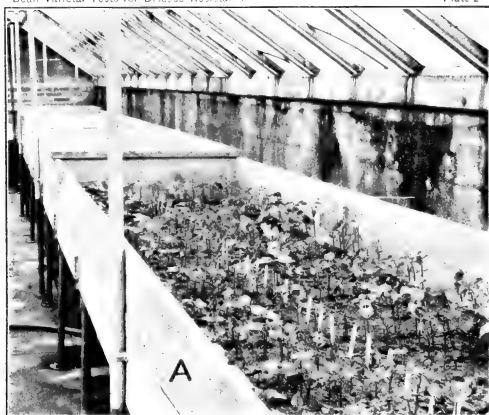
GREENHOUSE STUDIES

The greenhouse inoculation tests were conducted during the winters of 1922-23 and 1923-24 at Arlington Experiment Farm, near Rosslyn, Va., and were limited to anthracnose and bacterial wilt. Although considerable time has been given the problem, the writers have thus far failed to develop a satisfactory greenhouse method for obtaining quick and conclusive evidence on the relative susceptibility of varieties in the seedling stage to bacterial blight.

Since most of the American varieties have been tested by Barrus (1) for their separate reaction to the *alpha* and *beta* biologic forms of the anthracnose organism, principally foreign varieties were included in the authors' greenhouse studies. These were inoculated separately with the *alpha* form, and on account of the great similarity of the *beta* and *gamma* forms, as shown by the work of Burkholder (2), the comparative unimportance of the *gamma* form and limitations in greenhouse space these two forms were usually combined. All of the eight Minnesota forms were likewise combined, and to these were added in one inoculation (reported in Table III) the above-mentioned three forms from Cornell, the object being to obtain a control on the field results and, as already mentioned, to find varieties resistant to all known forms.

The different lots were planted in fresh loam soil in regular greenhouse benches with the sides raised 6 inches above the soil in order to turn them into moist chambers after inoculation by covering with wet burlap-lined coldframe sashes (pl. 2, A). Ten seeds of each variety were spaced 2 inches apart in short rows extending crosswise in the bench, and in every tenth row controls known to be susceptible to the biologic form being tested were planted. White Navy and Boston Pea were used most frequently for controls on *alpha*, Improved Golden Wax and ordinary Red Kidney for *beta*, and Well's Red Kidney, White Imperial, and Nova Scotia Marrow for *gamma*. The differential varieties and strains used as controls on the Minnesota biologic forms were planted in small pots and inoculated separately with each form in another greenhouse and then returned to spaces left vacant in the bench among the varieties to be tested, which in the meantime had been sprayed with a combined spore suspension of all the forms. In this way one could make sure that all cultures were virile and acting upon the varieties under the conditions of the test. The plants were inoculated as soon as possible after germination and kept moist by aid of the wet covering for 48 hours. The temperature ranged from 18 to 20° C. for the first few days, but later often went much higher. The results were recorded in all cases 10 days after inoculation. Plate 2, B is a typical illustration of the decisive outcome of experiments of this sort. From the above description it is seen that the temperature, time, and other conditions followed closely the standardized and very satisfactory procedure originally used by Barrus (1) and subsequently confirmed by the infection studies of Lauritzen (7).

The greenhouse wilt inoculations were made on plants grown in benches as above described and generally by the "cotyledon method." The temperature was held at about 25° C., and notes on permanent wilting of one or more of the first trifoliate leaves, as illustrated in Plate 3, B, were made one, two, and three weeks after



A.—View of a typical anthracnose inoculation experiment in the greenhouse, showing arrangement and size of plants at the time of inoculation. The farther end of the bench has already been sprayed with the inoculum and covered with wet burlap-lined sashes.

B.—Results of an anthracnose inoculation experiment on seedlings in the greenhouse, illustrating immunity and extreme susceptibility among five varieties of African beans. The varieties used in this test were planted Feb. 13, inoculated with a heavy spore suspension of the *alpha* biologic form of *Colletotrichum lindemuthianum* Feb. 26, and photographed 10 days later, Mar. 8, 1923.



B.—Results, 20 days after inoculation with the bacterial wilt organism in young epicotyls, at the second-leaf stage, of the Black Valentine variety. The general retardation of growth of two inoculated plants and permanent wilting of leaves are typical of the greenhouse infections reported in this paper



A.—A severe case of bacterial wilt developing on a nearly full grown plant in the field; most of the leaves are wilted or dead and the few pods are poorly filled, a condition illustrating the extent of infection defined as "severe" for this disease in Table I

inoculation. It soon became evident, however, that wilting alone was a poor index for comparison, since practically all plants inoculated showed this symptom. On account of more or less abnormal growth of both inoculated and control plants under ordinary greenhouse illumination in the winter, it was unfortunately not practicable to conduct the experiments for a longer period. A number of tests on potted plants carried for two months brought out much greater differences and showed that a longer experimental period is essential. The field seedling inoculations, therefore, gave a much better test of the ultimate effect of this disease on the plant than either the greenhouse seedling tests or the field inoculations on older plants.

WEATHER CONDITIONS AND PREVALENCE OF ANTHRACNOSE AND BLIGHT DURING THE PERIOD OF TEST

ANTHRACNOSE

During the entire period 1920 to 1923, inclusive, weather conditions were in general unfavorable for natural spread of anthracnose at both the Saginaw and East Lansing plots. At McMillan, Mich., on the other hand, epidemics developed during both years (1921 and 1922) that plots of American varieties of beans were maintained there. The usual midseason hot, dry spell at Lansing effectively checked the spread of the disease until the cool, moist weather of late summer. Conditions in 1920 and 1923 were not so unfavorable as to prevent the obtaining of abundant infection by repeated artificial inoculations, so that not only were epidemics produced in the two 1923 Michigan plots, but Doctor Leach reported satisfactory results in the duplicate established at St. Paul, Minn. A fairly decisive test was, therefore, obtained on all varieties planted in 1923.

BACTERIAL BLIGHT

Bacterial blight was generally prevalent and severe in all test plots during 1920, 1921, and 1923, and also on the late varieties following the extreme dry period of 1922. The disease was so destructive in parts of the Michigan bean section in 1921 that a number of fields of Red Kidneys were not harvested.

RECORDS OF DISEASE PREVALENCE

The data on the reaction of each variety to the various diseases, obtained from the different field plots during the entire period of test and from the greenhouse inoculations, have been critically reviewed and classified according to six standard grades of infection extending from very severe at the one extreme to none at the other. Brief definitions of these classes, which necessarily depend upon a different set of symptoms for each disease, are given in Table I.

Unfortunately sufficient knowledge of the diseases and refinement of technique are lacking to enable one to give a more definite expression of resistance or susceptibility than is shown by the headings of Table I. This is especially true in the case of bacterial wilt, where the progress of the disease under varying field conditions is poorly understood. Likewise difficulties are encountered in attempting to define clearly what appear to be intermediate degrees of susceptibility to bacterial blight. For mosaic, prevalence has been largely relied upon, although, as mentioned earlier, the data on this disease were obtained incidentally as they were available in the course of the other studies.

TABLE I.—Definitions of terms used to express the extent or degree of infection obtained from artificial inoculation and from natural spread of the different diseases

Disease	Extent of infection				
	Very severe	Severe	Moderate	Slight	Very slight
Anthracnose.....	Seedling usually dies. Lesions are deep and usually numerous on epicotyl and parts above. Pods become much spotted with deep lesions and rarely mature many seeds.	Seedling usually survives. Lesions are numerous on epicotyl, petioles, veins and pods and may be large and deep but usually do not seriously hinder development of most of the pods formed.	Seedling recovers from the infection. Lesions, although numerous on stems, leaves, and pods, not usually deep or extensive, and seed infection is rare.	Development of seedling apparently unaffected by the disease. Plant may show numerous lesions, but they are small in extent and mostly confined to ridges on stem, petiole, and to leaf veins.	Infection varies from small, black, definite lesions without spores to pale or brownish flecks.
Bacterial blight.	The plants appear yellow and burned and are largely defoliated with extensive lesions also on stems and pods which dry up with but few normally developed seeds. Seed infection may occur.	Leaves show many large spots, some yellowing and burning, but remain largely on the plant until maturity. Pods have numerous spots, are often poorly filled, but succeed in maturing a fair proportion of the seeds. Seed infection may occur.	Leaf lesions generally small, quickly limited by the larger veinlets and, although causing pronounced yellowing in some cases, rarely result in extensive defoliation. Pod lesions are few, small and shallow.	Numerous small lesions are tardily formed, but apparently have no effect on development of plant nor on normal ripening of the pods.	Occasional very small brown spots or flecks appear on both leaves and pods, but almost entirely disappear with further growth of the plant.
Bacterial wilt....	Plant is either killed outright in the seedling stage or lingers from a few weeks to a month and a half, in a much dwarfed condition with most of the older leaves flaccid or dead and the younger leaves gradually becoming affected until finally the entire plant succumbs.	Entire plant is dwarfed with numerous partially wilted or dead branches or leaves. Pods are few and poorly filled and a portion of the seeds show a yellow bacterial layer beneath the seed coat.	Plant is noticeably dwarfed or, in case of pole varieties, has a distinct spindling and unhealthy appearance with shriveling and shedding of the older foliage. Many pods are normally developed and ripened, but may bear infected seed.	Plant as a whole apparently recovers, although occasional branches and leaves are wilted or dying, and a small proportion of the pods fail to fill out. Seed infection may occur even on apparently healthy parts.	Development of plant is apparently unaffected by the disease, although in the early stages of growth incipient or permanent wilting of one or more of the first leaves and occasional defoliation may occur. Seed infection infrequent.
Mosaic.....	75 to 100 per cent of the plants show severe dwarfing and failure to set many pods.	50 to 75 per cent of the plants show pronounced symptoms, as dwarfing and distortion of leaves.	25 to 50 per cent of the plants are noticeably affected.	10 to 25 per cent of the plants are noticeably affected.	1 to 10 per cent of the plants are noticeably affected.
					No evidence of the disease.
					No evidence of infection whatever.
					No evidence of infection whatever.

In case of anthracnose it will be noted that the writers have followed very closely the definitions adopted by Barrus (1, p. 593) as a result of his extensive studies of this disease. The terms express the general effect of the disease on the plant, which takes into consideration both the number and size of the lesions, although, as Leach (8) has recently pointed out, size of lesion is doubtless the more important index of resistance.

In Table II there are listed all the varieties and selections of *Phaseolus vulgaris* which have been tested and, in successive columns, their reaction to the different diseases as expressed by the summary terms defined in Table I.

In examining the detailed records positive evidence was necessarily given most weight. Negative evidence, or signs of resistance, was considered significant only after comparison with controls and the behavior of the variety in the greenhouse and in other plots in different years. Unfortunately many of the varieties have not been tested long enough or under the necessary conditions to make such comparisons possible, and final judgment must await additional information.

The experimental results for bacterial blight on many of the American varieties, as shown in the ninth column, are supported by extensive observations in seedsmen's trial grounds and in large field areas growing in Michigan, New York, and Vermont.

The first column devoted to bacterial wilt gives the results of seedling inoculations in the greenhouse, the figures expressing the percentage of the total number of plants which showed permanent wilting of one or more of the first trifoliate or true leaves. The second column gives the field record. Here the occasional figures represent those varieties which were inoculated by the "cotyledon method" and show the percentage of plants, based on a unit of 10, which either died from the effects of the disease or were so stunted as not to mature pods. The surviving plants generally also displayed varying degrees of dwarfing, but usually succeeded in ripening many of their pods. The words in this column expressing different degrees of infection refer to the inoculations on older plants by the needle-prick method, described in the preceding section.

EVIDENCE OF RESISTANCE

A review of Table II shows the comparatively small number of varieties which were not moderately or severely infected with both anthracnose and bacterial blight, the diseases given special attention in this study. A large proportion of those tested but one season and showing the extent of infection indicated by "slight" or "none" would doubtless also have proved more or less susceptible had they been tested under more favorable conditions for the development of disease.

Since the evidence on resistance to anthracnose in the case of a number of the varieties was based largely on their behavior in the field, these and a number of others concerning which the information in Table II is uncertain or lacking were subjected to a final greenhouse test with all the known biologic forms of the fungus. The detailed results of this experiment, which was carried out in triplicate, are presented in Table III.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans (*Phaseolus vulgaris* L.) to greenhouse and field inoculations with anthracnose (*Colletotrichum lindemuthianum*), bacterial blight (*Bacterium phaseoli*), and bacterial wilt (*Bacterium flaccumfaciens*), and records on the natural occurrence of mosaic

(Varieties found most resistant to anthracnose blight or wilt are shown in italics. See reference letters a, b, and c and footnotes)

AMERICAN VARIETIES

Variety num-ber	Seed and plant intro-duction number	Variety name or seed characteristics	Source (seedsmen or locality)	Years tested	Anthracnose		Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations		Green-house inocu-lations	Field inocu-lations	

22	Michigan White Wax	Ferry	1920-1923	do	do	Very severe	do	100	None	Do.
23	New Hardy Wax	Stump & Walter	1923	do	do	do	do	No data	Severe	Moderate.
24	Pencil Pod Black Wax	Rice	1920-1923	do	do	do	Very severe	do	Slight	Do.
25	Prolific Black Wax	Drer	1920-1923	do	do	do	Moderate	100	Severe	Severe.
26	Refugee Wax	Ferry	1920-1923	do	do	do	do	70	None	Very Severe.
27	Roger Improved Kidney Wax	Roger Bros	1923	do	do	do	Severe	No data	Severe	Slight.
28	Round Pod Kidney Wax	Rice	1920-1923	do	do	Severe	do	89	do	Severe.
29	Rust-proof Wax	Ferry	1921	do	do	do	do	No data	No data	Slight.
30	Sure Crop Wax	Rice	1920-1923	do	do	Very severe	do	90	None	Do.
31	Trucker's Reward	Bolgiano	1923	do	do	Severe	Moderate	No data	Severe	None.
32	Unvalled Wax	Ferry	1920-1923	do	do	Moderate	do	100	Moderate	Slight.
33	Vaughn's Monster Wax	Vaughn	1923	do	do	Very severe	Severe	No data	Severe	Moderate.
34	Wardwell	Rice	1920-1923	do	do	Severe	do	do	do	Severe.
35	Webber Wax	do	1920-1923	do	do	do	do	do	do	Do.
36	Yosemite	Henderson	1922-1923	do	do	do	do	100	Severe	Do.
<i>Dwarf varieties with green pods</i>										
37	Bayo	Morse	1920, 1922, 1923	No data	No data	Very severe	Severe	No data	Moderate	Slight.
38	Black Valentine	Rice	1920-1923	do	do	Severe	Very severe	do	None	Do.
39	Bountiful	do	1920-1923	do	do	Very severe	Severe	100	Slight	Do.
40	Burpee Stringless	do	1920-1923	do	do	do	do	100	Severe	Do.
41	Canadian Wonder	Fish	1920-1923	do	do	Severe	Very severe	90	Moderate	Moderate.
42	China Red Eye	Rice	1920, 1921, 1923	do	do	Very severe	Moderate	80	None	Do.
43	Dwarf Horticultural	do	1920-1923	do	do	do	Severe	100	do	Severe.
44	Fordhook Favorite	Burpee	1920, 1921, 1923	do	do	Severe	Moderate	63	Moderate	Do.
45	French's Horticultural	Rice	1922, 1923	do	do	Slight	Slight	78	Slight	Do.
46	Full Measure	do	1920, 1921, 1923	do	do	Very severe	Severe	100	do	Moderate.
47	Giant Stringless	do	1920-1923	do	do	Severe	Moderate	100	None	Severe.
48	Goddard Improved	do	1920, 1921, 1923	do	do	Moderate	Severe	100	do	Slight.
49	Hodson Greenpod	Ferry	1920, 1921, 1923	do	do	Severe	do	No data	do	Do.
50	Lady Washington	Morse	1920, 1922, 1923	do	do	do	do	100	do	Very severe.
51	Longfellow	Rice	1920, 1923	do	do	do	Very severe	80	do	Severe.
52	Low Champion	do	1920, 1921, 1923	do	do	Very severe	do	50	Slight	Severe.
53	Marrow, Nova Scotia	Cornell University	1920-1923	do	do	Severe	Moderate	No data	None	Do.
54	Marrow, White	Rice	1920-1923	do	do	do	Severe	75	do	Do.
55	Masterpiece	do	1920-1923	do	do	Very severe	Very severe	88	do	Slight.
56	Mohawk	do	1920, 1921, 1923	do	do	Severe	Severe	100	do	Moderate.
57	Navy Pea types: Blue-pod Navy	Saginaw, Mich.	1920	do	do	do	do	No data	No data	Severe.
58	Blue Pod, small white.	California	1920	do	do	No data	Moderate	do	do	Do.
59	Boston Pea	Rice	1920, 1921, 1923	do	do	Very severe	Severe	do	None	Do.
60	Dwarf White Navy	Ferry	1920, 1921	do	do	Severe	do	do	No data	Slight.
61	Early Navy	Will	1920, 1921	do	do	do	do	do	do	Severe.

* Resistant to bacterial wilt.

* Resistant to bacterial blight.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

AMERICAN VARIETIES—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose		Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations		Greenhouse inoculations	Field inoculations	
Biologic forms										
Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection								
	No data	No data	Very severe	Moderate	88	None	Severe.			
62		Dwarf varieties with green pods—Continued	Michigan station.	1923	do	do	Severe	100	do	Do.
63		Navy Pea types—Contd.	Vaughn.	1920, 1922, 1923	do	do	do	No data	No data	Do.
64		Early Wonder	Michigan	1920	do	do	do	do	do	Do.
65		Improved Navy	Burbank	1920	do	do	do	do	do	Do.
66		Navy Pea	Michigan	1920	do	do	do	do	do	Do.
67		Pea Bean or Tree	Will.	1920, 1921	do	do	Very severe	do	do	Do.
68		Pea Bean	Michigan	1920, 1921	do	do	Severe	do	do	Slight.
69		Pilot Navy	Farquhar	1920, 1921	do	do	Very severe	do	do	Severe.
70		Prolific Tree	Michigan station.	1923	do	do	do	do	do	Do.
71		Small White Navy	Saginaw, Mich.	1920	do	do	do	do	do	Severe.
72		do	do	1920	do	do	do	do	do	Do.
73		White Navy	Bolzano	1920, 1921	do	do	Very severe	100	do	Do.
74		Robust (Olaf Nelson selection)	Michigan station.	1922, 1923	do	do	Slight	No data	None	None.
75		Robust No. 40520	do	1923	do	Slight	Moderate	do	do	Do.
76		"Robust Pea"	Burpee	1923	do	Very severe	Severe	do	do	Severe.
77		Red Kidney	Rice	1920-1923	do	do	Very severe	61	do	Slight.
78		Red Kidney, Wells'	Cornell station.	1920-1923	do	Slight	Severe	No data	Severe	Do.
79		Red Valentine	Rice	1920-1923	do	Severe	do	do	do	Moderate.
80		Refugee, Extra Early	do	1920-1923	do	do	do	100	do	Slight.
		Refugee 1000-1	do	1920-1923	do	Slight	Slight	83	do	Very severe.
		Refugee, Roger selection	Roger Bros.	1921, 1922, 1923	do	Moderate	Slight to moderate.	60	do	Severe.

81	Round Six Weeks	Ferry	1920-1923	do.	do.	Very severe	Severe	95	Slight	Slight.
82	Tennessee Green Pod	do.	1920-1923	do.	do.	Moderate	do.	95	None	Do.
83	Turtle Soup	Henderson	1920, 1922, 1923	do.	do.	Very severe	Very severe	100	do	Severe.
84	Warwick	Clark	1922, 1923	do.	do.	Moderate	Severe	No data	do	Slight.
85	White Imperial	Lyons, N. Y.	1920, 1921, 1923	do.	do.	Very slight	Slight ^b	100	do	Severe.
86	White Kidney	Rice	1920-1923	do.	do.	Severe	Very severe	100	Slight	Moderate.
87	Wonder Forcing	Stump & Walter	1923	do.	do.	Moderate	Severe	No data	None	Slight.
88	Yellow Six Weeks	Ferry	1920-1923	do.	do.	Very severe	do.	78	do	Do.
<i>Pole varieties with wax pods</i>										
89	Baldwin Wonder	Ferry	1920, 1921, 1923	No data	No data	Moderate	Slight ^b	60	None	Slight.
90	Golden Cluster	Rice	1920-1923	do.	do.	Severe	Moderate	88	do	Severe.
91	Indian Chief	Ferry	1920, 1921	do.	do.	do.	Severe	No data	No data	Do.
92	Kentucky Wonder Wax	Rice	1923, 1923	do.	do.	do.	Moderate	44	None	Slight.
93	Mont d'Or	Farquhar	1920, 1921	do.	do.	Very severe	Very severe	No data	No data	Do.
<i>Pole varieties with green pods</i>										
94	Burger Stringless	Rice	1923	No data	No data	Severe	Severe	No data	Slight	Severe.
95	Cane Knife	do.	1920-1923	do.	do.	do.	do.	100	None	Do.
96	Creaseback	do.	1920-1923	do.	do.	do.	Moderate	100	do	Slight.
97	Cut Short	do.	1920, 1921, 1923	do.	do.	do.	do.	88	do	Severe.
98	Harlequin	do.	1920, 1921, 1923	do.	do.	Moderate	do.	100	do	Do.
99	Kentucky Wonder	Fish	1920-1923	do.	do.	Severe	Severe	66	do	Slight.
100	Lazy Wife	Rice	1920, 1921, 1923	do.	do.	Very severe	Moderate	60	do	Severe.
101	London Horticultural	do.	1920, 1921, 1923	do.	do.	Severe	Slight ^b	No data	do	Do.
102	McCaslan	do.	1923	do.	do.	Moderate	Moderate	70	do	None.
103	St. Louis Perfection White	Ferry	1920, 1921, 1923	do.	do.	do.	do.	No data	do	Slight.
104	St. Fiacre	Farquhar	1920, 1921	do.	do.	Severe	Severe	83	No data	Do.
105	Scotta (Striped Creaseback)	Rice	1920, 1921, 1923	do.	do.	Moderate	Moderate	33 ^c	None	Do.
106	Tennessee Wonder	do.	1923	do.	do.	Slight	do.	No data	do	Severe.
107	Worcester Mammoth	do.	1920-1923	do.	do.	Severe	Slight ^b	70	Slight	Do.

ARGENTINE VARIETIES

(Seed obtained through American Consular Service)

108	46491	Colorados (Arroyo Seco)	Santa Fe Province.	1920	No data	No data	None	Very severe	No data	Slight.
109	46494	Mendocinos	Mendoza Province.	1920	do	do	do	Slight	do	None.
110	46495	Salteño	Salta Province	1920	do	do	do	Very severe	do	Slight.
111	46162	Salteños	Buenos Aires	1920	do	do	do	do	do	Do.
112	46493	Sanjuanino	San Juan Province.	1920	do	do	do	Moderate	do	Do.

^cResistant to bacterial blight.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

BELGIAN CONGO VARIETIES
(Seed presented by J. Burt-Davy, Johannesburg, Transvaal)

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic	
					Greenhouse inoculations	Field inoculations			Green-house inoculations	Field inoculations		
						Biologic forms						
					<i>Alpha, extent of infection</i>	<i>Beta and gamma combined, extent of infection</i>	<i>Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection</i>	Extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Percentage of plants killed from seedling inoculations and extent of infection on older plants	Extent of natural infection	
113	48472-A	<i>Pole varieties</i> Long, slender tan beans with dark red stripes and markings (similar to No. 612 from Urundi). <i>Large, light brown beans with greenish brown stripes (some sporting).</i> Long, plump, purple mottled beans with dark ring. <i>Small, wine-red kidney beans with dark stripes (similar to 589 from Urundi).</i> <i>Light, kidney-shaped beans with violet to blue stripes (similar to 608 from Urundi).</i>	Elizabethville.	1922	No data	No data	None	Slight	No data	No data	Slight.	
114	48472-B		do	1922, 1923	None	Severe	Moderate	Moderate	79	do	Do.	
115	48472-C		do	1922, 1923	do	None	None	Very slight ^a	Slight ^b	90	80	Do.
116	48472-D		do	1922, 1923	Very severe	Severe	Severe	Moderate	Moderate	90	No data	Severe.
117	48472-E		do	1922, 1923	None	Very slight	Very slight ^a	Very slight ^a	Severe	89	100	Slight.
118	48472-F		do	1922, 1923	do	do	Slight	Slight ^a	Slight ^b	100	100	None.

BRAZIL

(Seed obtained through American Consular Service)

[illegible]

Resistant to anthracnose.

Resistant to bacterial blight.

LIBRARY
CEREAL INVESTIGATIONS

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

BRAZIL—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Field inoculations	Green-house inoculations	Field inoculations	Mosaic	
Biologic forms					Extent of infection	Percentage of seedlings with one or more leaves per-manently wilted	Percentage of plants killed from seedling inoculations and extent of infection on older plants	Extent of natural infection			
Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection									
157	46215	Riscado	Sao Paulo	1920	No data	No data	None	Severe	No data	Moderate.	
158	46257	do	do	1920	do	do	do	Moderate	do	Severe.	
159	46216	Roxo	do	1920	do	do	do	Very slight	do	Slight.	
160	46510	Salmaso	Para	1920	do	do	do	Moderate	do	Moderate.	
161	46066	Vermelho (red bean)	Santos	1920	do	do	do	do	do	Slight.	
162	46518	Vermelho	Para	1920	do	do	do	Severe	do	None.	
163	46511	Viuva alegre	do	1920	do	do	do	Moderate	do	Slight.	

CHILE

(Seed obtained through American Consular Service)

164	46195	Bayos	Antofagasta	1920	No data	No data	Slight	No data	No data	Severe.
165	46196	Burritos	do	1920	do	do	do	do	do	Do.
166	46197	Caballeros	do	1920	do	do	do	do	do	Do.
167	46198	Canarios	do	1920	do	do	do	do	do	Do.
168	46199	Coscorones	do	1920	do	do	do	do	do	Slight.
169	46200	Frutillas	do	1920	do	do	do	do	do	Do.
170	46201	Ovalitos	do	1920	do	do	do	do	do	Do.
171	46202	Triguitos	do	1920	do	do	do	do	do	Severe.
172	46203	Bayos	Cosechas	1920	do	do	do	do	do	Moderate.
173	46230	Caballeros	do	1920	do	do	do	do	do	Do.
174	46231	Coscorones	do	1920	do	do	do	do	do	Do.
175	46526	Small white beans	Punta Arenas	1920	do	do	Slight	do	do	Severe.
176	46527	Light brown beans	do	1920	do	do	do	do	do	Do.

177	46528	White and yellowish white beans (mixed).	do	1920	No data	do	do	do	do	Do.
178	46529	Yellow to brown beans (mixed).	do	1920	do	do	do	do	do	Moderate
179	46530	Grayish brown beans	do	1920	do	do	do	do	do	Slight.
180	51701	Light tan pea beans	Santa Ines	1921	do	do	Severe	do	do	None.

COLOMBIA

181	46015	Rojo	Barranquilla	1920	No data	No data	None	Slight.	No data	Slight.
182	46016	De Santander	do	1920-1923	Severe	None	Severe	Severe	None	Very se- vere.
183	46142	Reddish brown, black mottled beans.	Medellin	1920	No data	No data	No data	Moderate	No data	None.
184	46143	Reddish brown beans	do	1920	do	do	do	do	do	Do.
185	46144	Cinnamon brown beans	do	1920	do	do	do	Slight	do	Do.

CZECHOSLOVAKIA

(Seed from Arnost Bahlsen, Prague)

		<i>Dwarf varieties</i>								
186		Cukrová perlička	Prague	1923	No data	No data	Slight	Severe	No data	Slight.
187		Flageolet Chevrierovy	do	1923	do	do	Moderate	do	do	Do.
188		Flageolet Zlutohlski	do	1923	do	do	do	Moderate	do	None.
189		Holandské bílé	do	1923	do	do	Slight	Severe	do	Slight.
190		Mont D'or Zlutohlské	do	1923	do	do	Severe	Moderate	do	None.
191		Nejranější černé	do	1923	do	do	Moderate	Severe	do	Do.
192		Nevýčerpateľné	do	1923	do	do	Severe	do	do	Severe.
193		Non Plus Ultra	do	1923	do	do	Moderate	Moderate	do	None.
194		Pannonia	do	1923	do	do	Slight	do	do	Do.
195		Sara	do	1923	do	do	Moderate	do	do	Do.
196		Vosková datle	do	1923	do	do	Very severe	do	do	Do.
197		Voskova (pole type)	do	1923	do	do	Severe	do	do	Do.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

ECUADOR

(Seed presented by F. W. Gooding, American consul general)

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations	
	</										

ENGLAND

Variety number	<i>Dwarf varieties</i>	Source	Years tested	Anthracnose		Bacterial blight		Bacterial wilt		Mosaic
				Greenhouse inoculations	Field inoculations	Field inoculations		Greenhouse inoculations	Field inoculations	
206	Canadian Express	Carter	1923	No data	Slight	Severe	Severe	No data	None	None.
207	Canadian Glory, stringless	do	1923	do	Severe	Severe	Moderate	do	do	Do.
208	Canadian Wonder	Sutton	1921-1923	No data	No data	No data	Severe	63	Severe	Severe.
209	<i>Canadian Wonder, selected</i>	do	1923	No data	do	do	Severe	No data	20	Do.
210	Early Giant	do	1923	do	do	do	do	do	do	Moderate.
211	Early Prolific	Carter	1923	do	do	do	do	do	do	Slight.
212	Everbearing	Sutton	1921-1923	Severe	Very slight	do	do	do	Severe	Severe.
213	Evergreen	do	1921-1923	do	Severe	do	Very severe	100	None	Do.
214	Fifty Days	Carter	1923	No data	do	do	Moderate	100	do	Do.
215	Foreing	Sutton	1921-1923	Moderate	No data	do	do	No data	do	None.
216	Golden Butter	Carter	1923	No data	Moderate	do	Very severe	78	do	Moderate.
217	Golden Wax Pod	Sutton	1923	do	No data	Moderate	Severe	No data	do	Do.

218	Green Gem.....	do.....	1921-1923	Moderate	Moderate	do.....	100	do.....	Slight.
219	Long Podded Negro.....	do.....	1921-1923	No data	Severe	do.....	80	Slight	Severe.
220	Longword, reselected	Carter	1923	Moderate	No data	do.....	No data	do.....	Slight.
221	Magnum Bonum.....	Sutton	1921-1923	None	Severe	do.....	80	None	Do.
222	Magpie.....	Carter	1923	No data	No data	do.....	No data	do.....	Moderate.
223	Monster Negro.....	Sutton	1921-1923	do.....	Severe	Slight	78	do.....	Slight.
224	Ne Plus Ultra, selected	do.....	1923	do.....	No data	Severe	No data	do.....	Moderate.
225	Osborn Early Forcing	Carter	1923	do.....	do.....	do.....	do.....	do.....	Slight.
226	Perfection.....	Sutton	1921-1923	Moderate	Moderate	do.....	do.....	do.....	Do.
227	Plentiful.....	do.....	1921-1923	Severe	Severe	do.....	do.....	do.....	Moderate.
228	Perpetual.....	Carter	1923	No data	No data	Very severe	do.....	Severe	Do.
229	Premier.....	Sutton	1923	do.....	do.....	Severe	do.....	Slight	Slight.
231	Prolific Negro.....	do.....	1921-1923	Moderate	Severe	do.....	90	None	Moderate.
232	Reliance.....	do.....	1921-1923	Slight	Moderate	Moderate	90	do.....	Do.
233	Satisfaction.....	do.....	1921-1923	Moderate	do.....	do.....	67	do.....	Slight.
234	Sunrise.....	Carter	1923	No data	No data	Severe	No data	Moderate	Moderate.
235	Supernative.....	Sutton	1921-1923	Moderate	Severe	Moderate	70	None	Slight.
236	White Haricot.....	do.....	1921-1923	do.....	Moderate	Severe	100	do.....	Severe.
237	White Model.....	Carter	1923	No data	No data	Moderate	No data	do.....	Do.
<i>Pole varieties</i>									
238	Earliest of All.....	Sutton	1923	No data	No data	Very severe	No data	None	Moderate.
239	July Climbing.....	Carter	1923	do.....	do.....	do.....	do.....	do.....	None.
240	Mont D'Or, Golden Butter	do.....	1923	do.....	do.....	do.....	do.....	do.....	Moderate.
241	Princess of Wales.....	Sutton	1923	do.....	do.....	Very slight	do.....	do.....	None.
242	Reselected.....	Carter	1923	do.....	do.....	Severe	Moderate	do.....	Severe.
243	Tender and True.....	Sutton	1923	do.....	do.....	Slight	do.....	do.....	Do.

FRANCE

(Seed from Vilmorin-Andrieux et Cie, Paris)

[illegible]

Resistant to anthracnose.

Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

FRANCE—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic	
					Greenhouse inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations	Field data		
					Biologic forms			Extent of infection	Percentage of seedlings with one or more leaves and extent of infection on older plants	Extent of natural infection		
Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection										
<i>Dwarf varieties—Contd.</i>					No data.	No data.	Moderate.	Moderate.	No data.	None.	Slight.	
255		Coco marbré nain.	Paris.	1921-1923	do.	Severe.	Severe.	Severe.	71.	do.	Moderate.	Moderate.
256		Comtesse de Chambord	do.	1921-1923	do.	do.	do.	do.	100.	do.	Severe.	Slight.
257		d'Alger (beurre) noir nain.	do.	1921-1923	do.	do.	do.	do.	50.	do.	Severe.	Slight.
258		De Bagnolet.	do.	1921-1923	do.	do.	do.	do.	100.	do.	None.	Severe.
259		De Bagnolet à feuille d'Or-tie.	do.	1921-1923	Severe.	do.	do.	do.	100.	do.	Moderate.	Slight.
260		De Bagnolet vert.	do.	1921-1923	No data.	Moderate.	Moderate.	do.	75.	Severe.	Severe.	Slight.
261		De Soissons nain.	do.	1921-1923	No data.	No data.	do.	do.	89.	No data.	do.	Slight.
262		Du Bon Jardinier.	do.	1921-1923	do.	Moderate.	do.	do.	89.	do.	do.	Moderate.
263		Du Bouscat.	do.	1921-1923	do.	Severe.	Slight.	Moderate.	75.	do.	do.	do.
264		Du Perreux.	do.	1921-1923	do.	do.	Moderate.	do.	100.	do.	Slight.	do.
265		Empereur de Russie.	do.	1921-1923	do.	Very slight.	Slight.	do.	100.	do.	do.	do.
266		Extra-precoce de Fontenay	do.	1921-1923	do.	Severe.	Severe.	Severe.	100.	do.	do.	do.
267		Flageolet beurre (nain)	do.	1921-1923	do.	Moderate.	Moderate.	Moderate.	89.	do.	do.	do.
268		Flageolet blanc à longue cosse.	do.	1921-1923	None.	Very slight.	Slight *	Severe.	100.	70.	Moderate.	Moderate.
269		<i>Flageolet blanc extra.</i>	do.	1921-1923	No data.	Moderate.	Moderate.	do.	86.	Severe.	do.	do.
270		Flageolet Chevrier.	do.	1921-1923	do.	do.	do.	do.	100.	do.	Severe.	do.
271		Flageolet de Vitry blanc.	do.	1921-1923	do.	do.	do.	Very severe.	67.	100.	None.	Slight.
272		Flageolet jaune amélioré.	do.	1921-1923	do.	do.	Very slight.	Severe.	83.	do.	Slight.	do.
273		Flageolet Merveille de France.	do.	1921-1923	do.	do.	Slight.	do.	100.	do.	Severe.	Severe.
274		Flageolet nain hâtif à feuille gaulée.	do.	1921-1923	do.	Severe.	do.	Very severe.	89.	Moderate.	do.	do.
275		Flageolet nain Triomphe des chassais.	do.	1921-1923	do.	Slight.	do.	Severe.	89.	None.	Slight.	Slight.

276	Flageolet noir	do	1921-1923	do	Severe	do	100	do	do	Do.
277	Flageolet Roi des verts	do	1921-1923	Very severe	Very slight	do	89	Severe	do	Moderate.
278	Flageolet rouge	do	1921-1923	No data	Very severe	do	100	None	do	None.
279	Flageolet rouge de Vitry	do	1921-1923	do	Moderate	do	100	do	do	Slight.
280	Flageolet très hâtif d'Etampes	do	1921-1923	Severe	None	do	100	do	do	Do.
281	Gloire de Lyon	do	1921-1923	No data	Severe	Slight	75	Severe	do	Do.
282	Gros vert hâtif	do	1921-1923	do	do	do	100	Severe	do	Do.
283	Incomparable (<i>H. express</i>)	do	1921-1923	Severe	Moderate	do	100	do	do	Do.
284	Incomparable à grain vert	do	1921-1922	No data	Severe to moderate	No data	100	No data	do	None.
285	Jaune cent-pour-un	do	1921-1923	do	Very slight	Slight	0	Severe	do	Do.
286	Jaune de la Chine	do	1921-1923	do	Slight	do	50	Slight	do	Slight.
287	Jaune du Canada	do	1921-1923	do	Very severe	Moderate	100	None	do	Moderate.
288	Le Bleu	do	1921-1923	do	do	do	44	do	do	Slight.
289	L'Inépuisable	do	1921-1923	Severe	Very slight	Slight	22	Severe	do	Do.
290	Merveille de Paris	do	1921-1923	No data	Moderate	do	67	None	do	Do.
291	Metis (<i>H. Eclipse</i>)	do	1921-1923	Severe	Severe	Severe	100	Slight	do	Do.
292	Nain blanc hâtif sans par-chemin	do	1921-1923	No data	Moderate to none	Moderate	100	None	do	Moderate.
293	Nain blanc quarantain	do	1921-1923	do	Moderate	Slight	100	Slight	do	Slight.
294	Nain blanc Unique	do	1921-1923	Very severe	Slight	Severe	88	Severe	do	Moderate.
295	Nain de Lignereux	do	1921-1923	No data	Severe	Moderate	100	Moderate	do	Slight.
296	Nain jaune extra-hâtif	do	1921-1922	do	do	do	90	No data	do	None.
297	Nain <i>Lyonnais à grain blanc</i>	do	1921-1923	Moderate	None	Slight	88	do	do	Slight.
298	Nain Lyonnais à très longue cosse	do	1921-1923	do	do	Moderate	75	None	do	Do.
299	Nain <i>Mangéout extra-hâtif</i>	do	1921-1923	None	Very slight	Slight	70	do	do	Do.
300	Nain Mangéout Phenix	do	1921-1923	No data	No data	Moderate	No data	do	do	Do.
301	Nain Parisien	do	1921-1923	do	Slight	Slight	56	Slight	do	Moderate.
302	Nain Roi des Beurres	do	1921-1923	do	No data	Moderate	No data	Severe	do	Do.
303	Noir de l'Hermitage	do	1921-1923	do	Very severe	Slight	100	do	do	Slight.
304	Noir hâtif de Belgique	do	1921-1923	do	do	Very slight	90	None	do	Do.
305	Predome nain	do	1921-1923	do	Severe to moderate	Slight	86	do	do	Do.
306	Prince noir	do	1921-1923	do	Severe	Moderate	100	do	do	Do.
307	Princesse nain à grosse cosse	do	1921-1923	do	do	Severe	100	Slight	do	Slight.
308	Rouge d'Orléans	do	1921-1923	do	do	Moderate	57	Severe	do	Severe.
309	Sabre nain (<i>H. hâtif de Hollande</i>)	do	1921-1923	Very severe	None	do	90	None	do	Very severe.
310	St. Esprit	do	1921-1923	No data	Severe	do	91	Slight	do	Slight.
311	Shah de Perse	do	1921-1923	do	Very severe	Severe	83	do	do	Do.
312	Suisse blanc (<i>H. lingot</i>)	do	1921-1923	do	do	Slight	79	Severe	do	Do.
313	Suisse rouge	do	1921-1923	do	do	Moderate	77	None	do	Do.
314	Surpasse tout	do	1921-1923	do	No data	Severe	No data	Moderate	do	Moderate.
315	Très hâtif de Cholet	do	1921-1923	do	Severe	Moderate	100	None	do	Do.
316	Très nain precoce	do	1921-1923	do	Very severe	do	89	do	do	None.

* Resistant to bacterial wilt.

* Resistant to bacterial blight.

* Resistant to anthracnose.

343	Prédome à rames	do	1921-1923	No data	Moderate to slight	do	Moderate	100	do	Severe.
344	Princesse à rames	do	1921-1923	do	None	Slight	Severe	100	do	Do.
345	Quatre-à-quatre	do	1921-1923	Very severe	Severe	Moderate	do	78	do	Slight.
346	Rig	do	1923	No data	No data	do	do	No data	do	Do.
347	Rond blanc commun	do	1921-1923	Severe to slight	None	Severe	do	89	do	Do.
348	Riz à rames	do	1921-1922	No data	Severe	No data	do	100	No data	No data.
349	Rouge de Chartres	do	1921-1923	do	Slight	Severe	do	100	None	Slight.
350	Sabre, à très grande cosse	do	1921-1923	Very severe	None	do	do	80	do	Severe.
351	Zébré gris	do	1921-1923	No data	Very severe	Slight	Slight	0	do	Slight.

GERMANY

	<i>Dwarf varieties</i>									
352	Flageolet oder Pariser, rote	Beck & Co.	1921-1923	No data	No data	Moderate	Severe	No data	None	Slight.
353	Flageolet oder Pariser, weisse.	do	1921	do	do	No data	do	do	No data	No data.
354	Hamburger Markt	Sprechelsen	1923	do	do	Very severe	do	do	None	Severe.
355	Hinrichs Reisen	Beck & Co.	1921-1923	do	do	Moderate	do	do	do	Slight.
356	Kaiser Wilhelm	do	1921-1923	do	do	Severe	Very severe	do	do	Slight.
357	Krummschnabel	H a g e u. Schmidt.	1923	do	do	Moderate	Severe	do	Severe	Slight.
358	Londoner Markt	do	1923	do	do	Slight	Moderate	do	None	Do.
359	Nordstern	do	1923	do	do	Very severe	Very severe	do	Severe	Severe.
360	Osborn's Treib	do	1923	do	do	Moderate	Severe	do	do	Moderate.
361	Prinzess, doppelte holland	Sprechelsen	1923	do	do	Severe	Moderate	do	None	Severe.
362	Saxa fadenlose	Schultz	1923	do	do	Moderate	do	do	100	None.
363	Schlachtschwert	Beck & Co.	1921-1923	do	do	do	Severe	do	None	Do.
364	Wachs Dattel	do	1921-1923	do	do	do	Very severe	do	do	Slight.
365	Wachs Rekord	Sprechelsen	1923	do	do	Very severe	Moderate	do	do	None.
366	Zucker Butter Brech	Beck & Co.	1921-1923	do	do	Moderate	Very severe	do	do	Severe.
	<i>Pole varieties</i>									
367	Don Carlos	do	1921-1923	do	do	do	do	do	do	None.
368	Flageolet Wachs, weissen bohnen.	Schultz	1921-1923	do	do	Severe	do	do	do	Severe.
369	Hinrichs Reisen	Beck & Co.	1921-1923	do	do	Very severe	Moderate	do	do	Slight.
370	Juli	Schultz	1921-1923	do	do	do	Severe	do	Slight	Do.
371	Meisterstück	H a g e & Schmidt.	1923	do	do	do	do	do	None	Do.
372	Mont d'Or, Wachs	Schultz	1921-1923	do	do	do	Very severe	do	do	Severe.
373	Mulscooper	H a g e & Schmidt.	1923	do	do	do	Severe	do	do	Slight.
374	Ohnegleichen	do	1923	do	do	do	Moderate	do	do	None.
375	Phänomen	Beck & Co.	1921-1923	do	do	Slight	Severe	do	do	Moderate.

* Resistant to bacterial blight.

* Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.
GERMANY—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic	
					Greenhouse inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations	Mosaic		
					Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Extent of infection	Percentage of plants killed from seedling with one inoculation or more leaves permanently wilted	Extent of natural infection		
376		Pole varieties—Continued Präsident. Rheinische Schmalz grosse, weisse. Schlachtschwert, allergrösste, weisse. Zehnwochen.	Schultz Beck & Co.	1923 1921-1923 1921-1923 1921-1923	No data	No data	Moderate	No data	None	None.		
377	do				do	Slight	do	do	Slight.			
378	do				do	Very severe	do	do	do	do	do	Do.
379	do				do	do	do	do	do	do	do	Very severe.

GUATEMALA

(Seed presented by Direccion General de Agricultura, Guatemala City)

Variety number	Seed and plant introduction number	<i>Dwarf varieties</i>	Source	Years tested	Anthracnose		Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations		Greenhouse inoculations	Field inoculations	
380	53892	Colorado de gancho de suelo.	Chimaltenango	1922, 1923	None	Slight	Severe	89	Moderate	Slight.
381	53839	Color de café	Quezaltenango	1922, 1923	Very severe	Severe	do	38	do	None.
382	53786-A	Cuarenteno negro	Ciudad Vieja, Sacatepequez.	1922	No data	Slight	do	No data	No data	Do.
383	53793	Negro	San Martín, Chimaltenango, Chimaltenango	1922	do	do	Moderate	do	do	Do.

53776	5384	Negro de vara	Sipacapa, San Marcos.	1922	do	do	None	Slight	do	do	Slight.
53784	5385	Negro pequeño mata (mixed), (A) <i>Very small, dull black pea beans.</i> (B) <i>Smaller shiny black beans.</i>	Cuilapa, Santa Rosa.	1922	do	do	do	Very severe	do	do	None.
53822	5386	Pinto negro sotero	do	1923	Very slight	None	Slight *	Moderate	74	50	Slight.
53840	5387	Puano de suelo (similar to 391).	do	1923	No data	No data	Moderate	do	No data	10	Do.
53823	5388	Pintillo	Acatenango, Chimalteango.	1922, 1923	Severe	Moderate	do	Severe	70	Severe	Do.
53826	5389	Pinto	Parramos, Chimalteango.	1922, 1923	None	Very severe	Severe	do	100	do	Do.
53829	5390	Pinto oscuro	Pastorez, Sacatepequez.	1922	No data	No data	do	do	No data	No data	None.
53821	5391	Rifon	San Lucas, Sacatepequez.	1922, 1923	None	Severe to moderate.	do	Moderate	70	Severe	Do.
53772	5392	Very small, slender black beans.	Quezaltenango	1922, 1923	Severe	Severe	do	do	83	do	Slight.
56584	5393	<i>Alubia (white kidney bean)</i>	Duenas, Sacatepequez.	1922, 1923	Moderate	Moderate	Slight	Severe	78	Slight	None.
53800	5394	<i>Pole varieties</i>	San Sebastian, Huehuetenango.	1922, 1923	Very severe	None	Moderate	do	86	None	Severe.
53801	5395	Amarillo	Orig. from Zamora, Spain.	1922	No data	No data	Slight	Very slight	No data	do	Slight.
53803	5396	do	San Lucas, Sacatepequez.	1922	do	do	do	Slight	do	do	Slight.
53804	5397	do	San Juan A., Huehuetenango.	1922	do	do	Very severe	Severe	do	do	None.
53880	5398	Amarillo de milpa	Ixtapacal San Rafael, San Marcos.	1922	do	do	Slight	Slight	do	do	Do.
53889	5399	Blanco	San Martin, Chimalteango.	1922	do	do	do	do	do	No data	Slight.
53876	5400	Blanco de enredo	San Mateo, Sacatepequez.	1922	do	do	None	do	do	do	None.
53876	5401	Blanco de suelo	La Candelaria, Barillas, Huehuetenango.	1922	do	do	do	do	do	do	Slight.
53876	5402	Blanco de suelo	Camalapa, Chimalteango.	1922	do	do	Slight	do	do	do	Very severe.

c Resistant to anthracnose

Resistant to bacterial blight.

Resistant to bacterial wilt

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

GUATEMALA—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose		Bacterial blight	Bacterial wilt		Mosaic	
					Greenhouse inoculations	Field inoculations		Green-house inoculations	Field inoculations		
					Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Percentage of plants killed from seedling inoculations and extent of infection on older plants	Field data	
					No data	No data	Slight	Severe	No data	No data	Severe.
403	53874	<i>Pole varieties</i> —Continued Blanco de vara	Santa Maria de J., Sacatepequez.	1922	None	None	Very slight ^a	do	80	10 ^c	Slight.
404	53875	Blanco de vara	San Rafael, San Marcos.	1922, 1923	No data	No data	None	Slight	No data	No data	Do.
405	53883	Blanco enrededor	Chimaltenango	1922	do	do	do	do	do	do	Severe.
406	53886	Blanco leandor	Parramos, Chimaltenango.	1922	do	do	do	do	do	do	
407	53887	Colima	San Rafael, San Marcos.	1922	Moderate	None	Slight	Moderate	89	90	Slight.
408	53781	Colorado	San Andres C., Sacatepequez.	1922, 1923	No data	No data	None	do	No data	No data	Moderate.
409	53888	Colorado camalapa enrededor.	Chimaltenango.	1922	do	do	Severe	do	do	do	Severe.
410	56583	De Mantua (dwarf type), originally from Valencia, Spain.	Guatemala City.	1923	do	do	None	Slight	do	do	Slight.
411	53786-B	Cuarenteno negro	Ciudad Vieja, Sacatepequez.	1922	Severe	Severe to none.	Severe	Severe	100	Slight.	Do.
412	53795	De riego mateado	Aguacatan, Huehuetenango.	1922, 1923	None	None	Slight ^a	Moderate	100	30 ^c	Do.
413	53792	De sarco	Zacualpa, Quiche.	1922, 1923							

53814	Gato enrededor.....	Patzum, Chimaltenango.	1922.....	No data.....	No data.....	Slight.....	No data.....	No data.....	Do.
53838	Ixcaco enrededor.....	Parraños, Chimalteango.	1922.....	do.....	do.....	Slight.....	do.....	do.....	Do.
53816	Kinak-Shak.....	Santiago, Sacanoria, Sacatepequez.	1922.....	do.....	do.....	do.....	do.....	do.....	Severe.
53791	Negro.....	Vaina morada, Chimalteango.	1922.....	do.....	do.....	do.....	do.....	do.....	Slight.
53766	do.....	Santa Catarina B., Sacatepequez.	1922.....	do.....	do.....	None.....	do.....	do.....	None.
53778	Negro colas enrededor.....	Patzum, Chimaltenango.	1922.....	do.....	do.....	do.....	do.....	do.....	Slight.
53787	Negro cuarenteno.....	San Pedro La Laguna, Solola.	1922.....	do.....	do.....	do.....	do.....	do.....	Do.
53789	Negro de mata.....	Barillas, Huehuetenango.	1922, 1923.....	Moderate.....	None.....	Slight.....	77.....	None.....	Do.
53783	Negro de milpa.....	San Martín, Chimalteango.	1922.....	No data.....	No data.....	None.....	No data.....	No data.....	Do.
53774	Negro de Suelo.....	San Andrés C., Sacatepequez.	1922.....	do.....	do.....	do.....	do.....	do.....	Do.
53775	Negro de vara.....	San Rafael, San Marcos.	1922, 1923.....	Very slight.....	None.....	Slight ..	100.....	100.....	Do.
53773	Negro enrededor.....	Chimalteango.	1922.....	No data.....	No data.....	do.....	No data.....	No data.....	None.
53770	do.....	Barillas, Huehuetenango.	1922, 1923.....	do.....	do.....	do.....	do.....	do.....	Moderate.
53771	Negro enrededor nebai.....	Quiche.....	1922.....	do.....	do.....	Severe.....	do.....	No data.....	Slight.
53777	Negro matedo.....	Aguacatan.....	1922.....	do.....	do.....	None.....	do.....	do.....	None.
53785	Negro pequeño.....	Japalmico, San Marcos.	1922, 1923.....	Severe to slight.....	None.....	Slight to moderate.....	71.....	70.....	Severe.
53873	Pequeño café.....	Tajumulco, San Marcos.	1922.....	No data.....	No data.....	Slight.....	No data.....	No data.....	Do.
53832	Plato Lengua de vaca.....	San Martín J., Chimalteango.	1922.....	do.....	do.....	Severe.....	do.....	do.....	Slight.
53872	Piloy.....	Xenacoj, Sacatepequez.	1922.....	do.....	do.....	do.....	do.....	do.....	Do.
53807	Pilique colorado.....	Senacoj, Sacatepequez.	1922, 1923.....	Moderate.....	None.....	Slight.....	90.....	50.....	Do.
53834	Pinto.....	San Mateo Ma, Sacatepequez.	1922.....	No data.....	No data.....	Severe.....	No data.....	No data.....	Severe.

- Resistant to anthracnose.

Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.
GUATEMALA—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsmen or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations			Greenhouse inoculations	Field inoculations	
						Biologic forms					
					<i>Alpha</i> , extent of infection	<i>Beta</i> and <i>gamma</i> combined, extent of infection	<i>Alpha</i> , <i>beta</i> , <i>gamma</i> , and I to VIII, inclusive, combined, extent of infection	Extent of infection	Percentage of plants killed from seedlings with one or more leaves permanently wilted	Extent of natural infection	
435	53831	<i>Pole varieties</i> —Continued Pinto de vara (mixed)-----	Neuvo Progreso, San Marcos.	1922-----	No data-----	No data-----	Slight-----	Moderate-----	No data--	None.	
436		(A) "Vara" type, small plump purple, stippled, with black stripes.	do-----	1923-----	Very severe-----	Severe-----	Moderate-----	Very severe-----	80-----	Slight.	
437		(B) Long, slender, black seed type.	do-----	1923-----	do-----	do-----	Slight-----	Severe-----	100-----	Moderate.	
438	53830	Pinto enrededor-----	Lemon Salenor, Chiantipa.	1922-----	No data-----	No data-----	None-----	do-----	No data--	Very severe.	
439	53828	Retinto del Suelo.	Jutiapa.	1922, 1923-----	Severe-----	Slight-----	Moderate-----	Moderate-----	60-----	Severe.	
440	53837	Rosado enrededor-----	do-----	1922, 1923-----	Very severe-----	Moderate to none.	Severe-----	do-----	90-----	Slight.	
441	53825	Sardo-----	San Mateo Ma, Sacatepequez.	1922-----	No data-----	No data-----	Slight-----	Severe-----	No data--	Do.	
442	53820	Varitas-----	San Antonio A. C., Sacatepequez.	1922, 1923-----	Severe to slight.	None-----	do-----	do-----	89-----	Severe.	
443	53764	Small black pea beans-----	Santo Tomas, Chichasatenango, Quiche	1922-----	No data-----	No data-----	None-----	Slight-----	No data--	Slight.	

			1922	do	do	do	Very slight	do	do	Do.
444	53765	Large black pea beans	San Antonio S., San Marcos, Miguel, Quiche.	do	do	do	do	do	do	Do.
445	53767	Very small, flat black beans	San Pedro las H., Sacatepequez.	do	do	do	do	do	do	None.
446	53768	do	San Pedro las H., Sacatepequez.	do	do	do	do	do	do	Slight.
447	53769	Small, flat black beans	San Pedro La Laguna, Solola.	do	do	do	do	do	do	None.
448	53794	Very small, flat black beans.	Huehuetenango.	do	do	Severe	Moderate	do	do	Slight.
449	53805	Small, black and tan beans (mixed).	Chimaltenango.	do	do	Slight	Severe	do	do	None.
450	53782	Round, black, red, and tan beans (mixed).	Chimaltenango, Chimaltenango.	do	do	do	Slight	do	do	Do.
451	53808	Small, red kidney beans	Aguaacatan, Huehuetenango.	None	None	Very slight ^a	do. ^b	88	40	Very severe.
452	53815	Large, chestnut-brown pea beans.	San Sebastian, Huehuetenango.	No data	No data	Severe	Moderate	No data	No data	Slight.
453	53827	Mottled tan kidney beans with dark stripes.	Chimaltenango.	do	do	do	Severe	do	do	Severe.
454	56582	Light tannish-pink beans	Originally from Mexico.	do	do	do	Very slight ^b	do	None	Very severe.
455	53817	White pea beans	Colotenango, Huehuetenango.	Very slight	None	Very slight ^a	Moderate	100	10	Severe.
456	53842	Small white kidney beans.	Unknown	No data	No data	Slight	Severe	No data	No data	Very severe.
457	53877	White pea beans	San Pedro La Laguna, Solola.	do	do	Severe	Slight	do	do	Slight.
458	53878	do	Acatenango, Chimaltenango.	do	do	do	do	do	do	Severe.
459	53882	Small white pea beans	do	do	do	Slight	do	do	do	Slight.
460	53884	Small white marrow	Zaragoza, Chimaltenango.	do	do	do	Severe	do	do	None.
461	53885	do	Itzapa, Chimaltenango.	do	do	None	Slight	do	do	Slight.
462	53890	White marrow	San Antonio S., San Marcos.	do	do	do	do	do	do	Do.
463	56585	Large white marrow, Bahama beans.	Guatemala City.	do	do	do	Very severe	do	None	None.

^a Resistant to anthracnose.^b Resistant to bacterial blight.^c Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

HOLLAND

(Seed from Sluis & Groot, Enkhuizen)

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose		Bacterial blight	Bacterial wilt		Mosaic	
					Greenhouse inoculations	Field inoculations		Greenhouse inoculations	Field inoculations		
Biologic forms		Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Extent of infection	Percentage of plants killed from seedling inoculations and extent of infection on older plants				
464		<i>Dwarf varieties</i>		1923	No data	No data	None	Moderate	No data	None	Slight.
465		Best of All, speckled seeded, stringy.		1923	do	do	Slight	do	do	do	Do.
466		Best of All, speckled seeded, stringless.		1923	do	do	do	do	do	do	Very severe.
467		Best of All, white seeded, stringy.		1923	do	do	None	do	do	do	Slight.
468		Best of All, white seeded, stringless.		1923	do	do	Slight	do	do	do	Do.
469		Dark Dun or Liver Color, stringy.		1923	do	do	do	do	do	do	Very severe.
470		Delicata, stringless.		1923	do	do	do	do	do	do	Slight.
471		Dutch Middle Broad Longpod, stringy.		1923	do	do	Moderate	Severe	do	do	Do.
472		Early White Farming, stringy.		1923	do	do	Slight	do	do	Slight	Severe.
473		Extra Large Sword or Caseknife, stringy.		1923	do	do	Moderate	do	do	None	Very severe.
474		Extra Large Sword or Caseknife, stringless.		1923	do	do	do	do	do	do	Do.
475		North Star		1923	do	do	do	do	do	do	Moderate.
476		Pale Dun or Cream Color, stringy.		1923	do	do	do	Moderate	do	do	Slight.
477		Perfect, dwarf white early, stringless.		1923	do	do	Slight	Severe	do	do	Very severe.
		Princess White, stringy		1923	do	do	Very slight	Very severe	do	Moderate.	Severe.

478	Princess White, stringless	1923	do	do	Slight	Severe	do	None	Very severe.
479	Transvalian, stringless	1923	do	do	do	do	do	do	Do.
480	Volger Edible Podded White, stringless.	1923	do	do	Very severe	Very severe	do	Slight	Do.
	<i>Pole varieties</i>								
481	Abundance Dutch Sword, stringy.	1923	No data	No data	Very severe	Severe	No data	None	Severe.
482	Avant Garde, stringy	1923	do	do	do	do	do	do	Do.
483	Perfection, finest white pearl, stringless.	1923	do	do	Moderate	do	do	do	Moderate.
484	President Roosevelt, stringless.	1923	do	do	Very severe	do	do	do	Severe.
485	Princess Small White, stringless.	1923	do	do	Moderate	do	do	do	Do.
486	Vancelots Giant White Princess, stringless.	1923	do	do	Severe	do	do	do	Do.

HONDURAS

(Seed from Bally Tree Falls, British Honduras)

	<i>Pole varieties</i>								
487	De Milpa	1922	No data	No data	Slight	Slight	No data	No data	Slight.
488	do	1922	do	do	None	No data	do	do	None.
489	Pelon (small yellow)	1922	do	do	do	None	do	do	Do.

ITALY

(Seed from Fratelli Ingegnoli, Milan)

	<i>Dwarf varieties</i>								
490	Borlotto screziato O Regina.	1923	No data	No data	Severe	Moderate	No data	Slight	Slight.
491	Butirro bianco commune.	1923	do	do	do	do	do	None	Very severe.
492	Cento per uno.	1923	do	do	do	Severe	do	do	Slight.
493	Da Cornetto giallo.	1923	do	do	Moderate	Moderate	do	do	Do.
494	Meraviglia di Lione	1923	do	do	do	Slight	do	Slight	Do.
495	Nostrano Aquila	1923	do	do	do	Moderate	do	Severe	Do.
496	Quarantino bianco, "Cannellino."	1923	do	do	Slight	do	do	None	Do.

* Resistant to bacterial blight.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.
ITALY—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose		Bacterial blight		Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations		
Biologic forms		Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Extent of infection on older plants						
Alpha, extent of infection	Beta and gamma combined, extent of infection										
497	498					499	1923	1923	1923	No data	No data
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923	1923	do	do	do	do	do	Do.
			1923	1923							

512	Sarasa Ingen	do	1921-1923	do	do	Severe	do	do	do	Do.
513	Stringless Wax pod	do	1921	do	do	None	do	do	No data	None.
	<i>Pole varieties</i>									
514	Chufuku	M. F. Barrus, Cornell University.	1920	No data	No data	None	Severe	No data	No data	Severe.
515	Chunagazura	Direct importation.	1923	do	do	Moderate	Moderate	do	None	Moderate.
516	Chunagazura	M. F. Barrus, Cornell University.	1920	do	do	None	Severe	do	No data	Slight.
517	Chu Tenashi	do	1920	do	do	do	do	do	do	Severe.
518	Diafuku, large white butter.	do	1920	do	do	do	do	do	do	Moderate.
519	Kintoki (Low's Champion)	do	1920	do	do	do	do	do	do	Slight.
520	Kotenshi, small white pea	do	1920	do	do	do	do	do	do	Severe.
521	Kumamoto Ingen	do	1920	do	do	do	do	do	do	Do.
522	Muro Ingen	do	1920	do	do	do	do	do	do	Very severe.
523	Nagazura (Goddard)	Direct importation.	1923	do	do	Moderate	Moderate	do	None	None.
524	Naga Usura, mottled kidney.	M. F. Barrus, Cornell University.	1920	do	do	None	do	do	No data	Slight.
525	Ossaya Dais Ingen	Oriental Seed Co.	1921-1923	do	do	Slight	Severe	do	None	Severe.
526	Otafuku, white	do	1921	do	do	None	do	do	No data	Slight.
527	Shiro Tsurumashi	do	1921	do	do	do	Moderate	do	do	Do.
528	Suzunari Ingen	do	1921-1923	do	do	Slight	Slight	do	None	None.
529	Yatsubusa, black seed	do	1921-1923	do	do	Moderate	Severe	do	do	Severe.
530	Yatsubusa, brown seed	do	1923	do	do	Severe	Moderate	do	do	None.
531	Yatsubusa, white seed	do	1921-1923	do	do	Moderate	Severe	do	do	Very severe.

JAVA

(Seed presented by C. J. J. Van Hall)

532	<i>Pole variety</i>	Departement Landbouw.	1923	Moderate	Moderate	None	Moderate	No data	None	Slight.
	Katjang merah									

* Resistant to bacterial blight.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.
KENYA COLONY, AFRICA
(Seed collected by H. L. Shantz)

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations		
Biologic forms			Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Percentage of plants killed from seedling inoculations and extent of infection on older plants			
533 534 535	51528 52205 52206	<i>Dwarf varieties</i> Red beans..... Brown beans with maroon stripes and flecks. Small, shiny, and dull black beans (similar to Nos. 573 and 649 from Urundi). Large, narrow, red beans. Long, narrow, light brown beans with darker stripes and flecks.	Meru District, Nairobi. Kisumu.....do.....	1921..... 1921..... 1921-1923.....	No data.....	No data.....	None.....	Severe.....	No data.....	No data.....	Slight.
					do.....	do.....	Severe.....	Very severe.....	do.....	Do.	
					Very severe.	Very slight.....	do.....	Severe.....	100.....	Very severe.	
536 537	52207 52209do.....do.....do.....do.....	1921..... 1921.....	No data.....	No data.....	None.....	Very severe..... Moderate.....	No data..... do.....	No data..... do.....	Do. None.

MEXICO
(Seed presented by E. J. Hands, Dos Cebezos, Ariz.)

538	53532	<i>Pole variety</i> Large tan beans with choo- late-colored stripes.	Chihuahua.....	1922.....	No data.....	None.....		No data.....	No data.....	Severe.
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PERU

(Seed presented by Luis Roos & Co., Callao-Lima)

	<i>Pole varieties</i>	Pacasmayo-Chincha	1920-1923 1920	Very severe. No data	Severe. No data	Moderate. None	Severe. Slight	78 No data	None. Do.
539	Panamitos								
540	Negros								

RUSSIA

(Seed presented by J. Palibin, Director Botanic Garden, Batum, Transcaucasia)

	<i>Pole varieties</i>	Kutais district. Georgia district.	1921	No data	No data	None	Slight ^b	No data	None.
541	Short, thick beans with marmoon spots on white background.								
542	Light-tan beans with lilac spots.								
543	Dark-tan beans with lilac spots.								
544	Grayish-tan beans with dark ring around hilum.								
545	Brownish-tan beans with dark ring around hilum.								
546	Long, slender, dark-red beans.								

UGANDA

(Seed collected by H. L. Shantz)

	<i>Dwarf varieties</i>	Misindi	1921-1923	Severe.	Severe.	Severe	Moderate	86	Severe.
547	Short lavender beans with dark ring.								
548	Short drab beans with dark ring.								
549	(A) Short light brown beans with dark ring.								
550	(B) Long, kidney-shaped dark brown beans with dark ring.								
551	Short light green beans with brown ring.								
552	Short greenish-tan beans with brown ring.								

^a Resistant to anthracnose.^b Resistant to bacterial blight.^c Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

UGANDA—Continued											
Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt	Mosaic	
					Greenhouse inoculations	Field inoculations	Field inoculations	Field inoculations	Greenhouse inoculations		
					Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection	Extent of infection	Percentage of seedlings with one or more leaves permanently wilted	Percentage of plants killed from seedling inoculations and extent of infection on older plants	
553	52194	<i>Dwarf varieties</i> —Contd.	Misindi	1921-1923	Very slight	Severe	Slight	Severe	100	Severe	Very severe.
554	52196	Long yellowish-tan beans with dark ring.	do	1921-1923	None	Moderate	Moderate	Moderate	59	100	Slight.
555	52197	Large long red beans (probably identical with above).	do	1921-1923	do	Severe	Slight	do	87	90	Do.
556	52198	<i>Small light pink beans with stripes and flecks of maroon.</i>	do	1921	No data	No data	do	Slight	No data	No data	None.
557	52199	Small flat dull-tan beans	do	1921-1923	Very severe	Moderate	Severe	Moderate	63	Moderate	Slight.
558	52186	<i>Pole varieties</i>									
559	52187	Small black beans	Misindi	1921	No data	No data	None	Moderate	No data	No data	None.
560	52188	Long maroon beans with cream flecks.	do	1921	do	do	do	do	do	do	Do.
561	52195	Blue and gray stippled kidney beans with dark ring around hilum.	do	1921-1923	Very severe	Severe	Severe	Severe	90	do	Slight.
562	52200	Long tan beans with brown ring.	do	1921	No data	No data	None	Moderate	No data	No data	None.
563		(A) Light-tan beans with dark purple stripes.	do	1921-1923	Very severe	Very severe	Very severe	do	100	None	Slight.
		(B) Gray beans with olive green stripes and brown ring.	do	1923	do	Moderate	Severe	Severe	100	do	Severe.
564		(C) Gray beans with scattered brown stippling and brown ring.	do	1923	do	do	No data	No data	100	No data	No data.

565	52201	Long cream beans with dark red mark through hilum.	do.	1921	No data	No data	Severe	Slight ^b	No data	do.	None.
566	52202	Long, broad white beans	do.	1921	do	do	Slight	Severe	do	do	Do.
567	52203	Small, short white beans	do.	1921-1923	Very severe	Very severe	Very severe	Very severe	91	None	Severe.

URUGUAY

(Seed presented by D. Basso, through American Consular Service)

568	46164	De la Regna (originally from Italy).	Montevideo	1920	No data	No data	None	Moderate	No data	No data	Slight.
569	46165	Agulla (originally from Italy).	do.	1920	do	do	do	Severe	do	do	Do.

URUNDI, AFRICA

(Seed collected by H. L. Shantz)

<i>Pole varieties (except Nos. 574 and 615)</i>											
570	50251	Somewhat flattened white pea bean.	Usumbura	1921-1923	Very severe	None	Moderate	Moderate	93	None	Very severe.
571	50252	Large greenish yellow kidney beans.	do.	1921	No data	No data	None	Severe	No data	No data	None.
572	50253	Short light brownish beans.	do.	1921-1923	None	Severe	Moderate	do	83	70	Slight.
573	50254	(A) Small slender black kidney beans (similar to Nos. 535 and 649 from Kenya).	do.	1921-1923	Severe	None	Severe	do	80	None	Severe.
574		(B) Small short sooty-brown mixture from above.	do.	1923	Very severe	do	Moderate	do	100	do	Very severe.
575	50256	Light yellow beans with dark stripes.	do.	1921	No data	No data	Slight	Moderate	No data	No data	Slight.
576	50257	Reddish beans with dark stripes.	do.	1921	do	do	None	do	do	do	Do.
577	50259	Large flat purple-mottled beans with dark ring.	do.	1921-1923	Very severe	do	Moderate	Slight ^b to moderate.	90	Slight	Moderate.
578	50260	Purple, red-mottled beans.	do.	1921	No data	do	None	Slight ^b	No data	No data	None.
579	50261	Deep-red beans.	do.	1921	do	do	do	Severe	do	do	Do.
580	50262	Deep-purple beans.	do.	1921	do	do	do	Moderate	do	do	Do.
581	50263	Gray beans.	do.	1921	do	do	do	do	do	do	Do.
582	50264	Short, plump, reddish beans with light-purple stripe.	do.	1921-1923	None	Severe	Severe	do	70	80	Very severe.

^b Resistant to bacterial blight.

		do	1921-1923	None	Very severe to none	None	Very slight ^a	Slight ^b	100	100	None
594	Short, plump, Sudan-brown beans (similar to No. 572, slight segregation).	do	1921-1923	None	Very severe to none	None	Very slight ^a	Slight ^b	100	100	None
595	Short, plump Brussels-brown beans (similar to Nos. 572 and 594).	do	1921-1923	Very severe to none	Very severe to none	None	Moderate	Moderate	94	80	Slight.
596	Long, plump, dark-tan beans (similar to No. 591, much segregation).	do	1921-1923	No data	No data	No data	Slight	Severe	No data	100	Do.
597	Purple-chocolate colored marrow beans (probably identical with 653).	do	1921-1923	None	None	None	Very slight ^a	Slight to moderate ^b	83	90	None.
598	Rich tan-colored bean with mottled surface.	do	1921-1923	do	Severe	Severe	Moderate	Severe	90	None	Slight.
599	Small wine-red kidney beans with dark stripes (similar to No. 117 from Belgian Congo).	do	1921-1923	do	Very severe	Very severe	do	do	81	82	Do.
600	Very small, plump olive-green beans with tan mottling.	do	1921-1923	Very severe	do	do	Severe	do	90	30 ^c	None.
601	Yellow beans with brown markings.	do	1921	No data	No data	No data	None	do	No data	No data	Do.
602	(A) Finely stippled wine-red kidney beans with black stripes.	do	1921-1923	None	Severe	Severe	Moderate	Moderate	100	None	Severe.
603	(B) Finely stippled lavender kidney beans with olive stripes.	do	1923	No data	No data	No data	None	do	No data	do	Slight.
604	Very small flat, tan beans with black stripes.	do	1921-1923	Very severe	Slight	Slight	Very severe	Severe	83	do	Severe.
605	(A) Light-tan beans with light olive-green stripes.	do	1921-1923	None	Severe	Severe	Slight	do	89	do	Slight.
606	(B) Tan kidney beans with olive stripes.	do	1923	do	do	do	Moderate	Moderate	83	do	Do.
607	(C) Long, finely stippled, blue-tan beans with black stripes (similar to No. 595).	do	1923	do ^a	Very slight ^a	Very slight ^a	No data	No data	89	No data	No data.
608	Light kidney-shaped beans with violet to blue stripes (similar to No. 118 from Belgian Congo).	do	1921-1923	do	Very severe	Very severe	Moderate	Severe	100	30 ^c	Slight.
609	Deep yellowish brown beans with black stripes.	do	1921-1923	do	do	do	Slight	Moderate	67	None	Do.
610	Flat, pinkish kidney beans with purple stripes.	do	1921-1923	do	do	do	Severe	do	95	65	Do.
611	Small, plump pinkish beans with purple stripes.	do	1921-1923	do	Severe	Severe	do	do	78	55	Do.

^a Resistant to anthracnose.^b Resistant to bacterial blight.^c Resistant to bacterial wilt.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

URUNDI, AFRICA—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight		Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Field inoculations	Greenhouse inoculations	Field inoculations			
								</				

		do	1921-1923	Severe	Slight	Slight	do	67	70	None.
623	50877	(A) Small, slender, grayish-tan beans with bluish-black mottling (much segregation).	do	Severe	Slight	Slight	do			
624		(B) Flat, olive-brown beans with tan mottling.	do	do	No data	Moderate	Moderate	100	50	Do.
625	50878	Long, mottled reddish-lavender beans with dark ring (similar to No. 630).	do	None	do	Slight	Severe	80	None	Slight.
626	50879	(A) Blue and gray stippled kidney beans (similar to No. 560 from Uganda).	do	do	Severe	Severe	Very slight ^b	100	do	Do.
627		(B) Flat, blue-tan mottled beans.	do	do	Very slight	Very slight ^a	Moderate	80	do	Do.
628	50880	Short, plump, tan-purple stippled beans with dark ring.	do	No data	No data	None	Severe	No data	No data	None.
629	50881	Small, purple-gray mottled beans with dark ring.	do	None	Severe	Moderate	Moderate	89	None	Severe.
630	50882	Long, mottled lavender beans with brown ring (similar to No. 625).	do	do	Very severe	do	do	100	do	Do.
631	50883	Small, flat, yellowish-tan mottled beans (some segregation).	do	do	Very slight	Slight ^a	Severe	89	50	Do.
632	50884	Long brown-mottled beans with dark ring.	do	do	Very severe	Moderate	do	100	None	Do.
633	50885	Purple - chocolate - colored marrow beans (probably identical with No. 597).	do	do	None	None ^a	Moderate	94	85	Slight.
634	50886	(A) Small, flat, maroon beans.	do	do	Severe	Moderate	Severe	100	None	Do.
635		(B) Small, flat, pink beans.	do	No data	None	do	Slight ^b	No data	do	Do.
636		(C) Small gray beans with red stippling on end or back.	do	Very slight	Severe	Slight	do ^b	89	do	Do.
637	50887	Short, plump, chocolate-brown beans (similar to No. 638).	do	None	Very severe	Severe	Severe	73	70	Do.
638	50888	(A) Light-chocolate-brown marrow beans (similar in plant characters to No. 635).	do	do	None	Very slight ^a to none.	Moderate	81	50	None.
639		(C) Small, flat, dark-tan beans.	do	No data	Slight	Slight	do	70	65	Slight.
640	50889	Long, plump, greenish-yellow beans with dark ring around hilum.	do	Moderate	Very severe	do	Very severe	79	None	Very severe.
641	50891	Small, nearly round, straw-colored beans.	do	Very severe	No data	do	do	90	do	Severe.
642	50892	Large, white kidney beans (very late).	do	do	Severe	Severe	Severe	75	do	Very severe.

^a Resistant to anthracnose.^b Resistant to bacterial blight.

TABLE II.—Summarized data on the reaction of some American and foreign varieties of beans to greenhouse and field inoculations—Contd.

URUNDI, AFRICA—Continued

Variety number	Seed and plant introduction number	Variety name or seed characteristics	Source (seedsman or locality)	Years tested	Anthracnose			Bacterial blight	Bacterial wilt		Mosaic
					Greenhouse inoculations	Field inoculations	Greenhouse inoculations		Field inoculations		
										Biologic forms	
Alpha, extent of infection	Beta and gamma combined, extent of infection	Alpha, beta, gamma, and I to VIII, inclusive, combined, extent of infection									
643	50893	<i>Pole varieties (except Nos. 574 and 619)—Continued</i> Large, white pea beans (very late).	Nyanza	1921-1923	None	Very severe	Moderate	Moderate	100	None	Slight.
644	50894	Small, white navy beans	do.	1921-1923	Very severe	None	do.	Severe	100	60	Severe.
645	50895	Long, white beans	Kigoma	1921	No data	No data	None	Moderate	No data	No data	Slight.
646	50896	Large, black pea beans	Nyanza	1921-1923	Severe	Very severe	Moderate	Severe	100	None	Moderate.
647	50897	Long, black beans	do.	1921-1922	No data	No data	None	do.	No data	No data	Slight.
648	50898	Long, flat, black beans, slightly longer than above.	do.	1921-1923	Moderate	Moderate	Slight	do.	67	None	Moderate.
649	50899	Very small, sooty-black beans (similar to No. 535 from Kenya Colony).	do.	1921-1923	Very severe	None	Very severe	do.	100	do.	Very severe.
650	50900	(A) <i>Light-brown beans with dark stripes.</i>	do.	1923	None	Slight	Slight ^a	Moderate	50	do.	Do.
651		(B) Long, flat, bluish-black beans.	do.	1921-1923	No data	Very severe	Moderate	Severe	56	do.	None.
652	50905	Long, narrow, mottled purplish beans with greenish stripes.	do.	1921	do.	No data	None	do.	No data	No data	Do.

VENEZUELA

(Seed presented by H. Pittier, Caracas)

	46363		1920	No data	No data	Severe	No data	No data	Very se- vere, Severe.
653	46363	Caraota blanca	Caracas	1920	No data	None	No data	No data	Very se- vere, Severe.
654	46369	Caraotas indieita pe- queña.	do	1920	do	do	do	do	
655	46366	Caraota negra	do	1920	do	do	do	do	Moderate.
656	46180	Caraotas bayas	Maracaibo	1920	do	do	do	do	Do.
657	46179	Caraotas negras	do	1920	do	do	do	do	Severe.
658	46181	Caraotas pintadas	do	1920	do	do	do	do	Severe.
659	46368	Guacamaya	Caracas	1920	do	do	do	do	Slight.
660	46370	<i>Guacararo colorado</i>	do	1920	do	do	do	do	None.
661	46365	<i>Guacararo redondo pintado</i>	do	1920	do	do	do	do	Moderate.
662	46364	Huevo de paloma	do	1920	do	do	do	do	Very se- vere.
663	46362	Poncha rosado	do	1920	do	do	do	do	Moderate.
664	46367	Poncha rosada jaspeada	do	1920	do	do	do	do	Slight.

^a Resistant to anthracnose.

Resistant to bacterial blight

TABLE III.—Detailed results of greenhouse inoculations with a combined spore suspension of all known biologic forms of *Colletotrichum lindemuthianum* on some of the more resistant varieties and on certain others concerning which the information in Table II is incomplete or lacking

Variety number	Name or seed characteristics	Source	Number of plants showing the extent of infection indicated						Remarks
			Very severe	Severe	Moderate	Slight	Very slight	None	
11	Dwarf Golden Carmen...	Roger Bros.					7		Apparently resistant.
115	Large, light-brown beans with greenish-brown stripes.	Belgian Congo.						20	Immune.
255	Coco marbre nain.....	France.....	10	20					Very susceptible.
273	Flageolet Mervielle de France.	do.....		1	11				Moderately susceptible.
286	Jaune de la Chine.....	do.....				7			Apparently resistant.
289	L'Inépuisable.....	do.....		10					Susceptible.
293	Nain blanc quarantain.....	do.....	1	21	5				Do.
301	Nain Parisien.....	do.....				15	5		Resistant.
325	Coco bicolore du Pape.....	do.....		22					Susceptible.
327	Coco blanc.....	do.....	1	5					Do.
336	Mangetout de la Vallée.....	do.....	5	9	7				Do.
339	Mangetout du Maine.....	do.....		8	4				Do.
344	Princess.....	do.....		4	15				Moderately susceptible.
375	Phänomen.....	Germany.....	2	10					Susceptible.
376	Präsident.....	do.....	16						Very susceptible.
377	Schmalzgrosse.....	do.....		9	1				Susceptible.
386	Negro pequeño mata (Strain A).	Guatemala.....			7	2		18	Very resistant.
404	Blanco de vara.....	do.....			1		14	4	Do.
408	Colorado.....	do.....			3	1	4	20	Do.
413	De sarco.....	do.....		1				24	Immune.
429	Negro pequeño.....	do.....		3	21				Moderately susceptible.
433	Pilique colorado.....	do.....			2	6		23	Very resistant.
442	Varitas.....	do.....			1	12	11		Resistant.
451	Small red kidney beans.....	do.....		2	8			23	Very resistant.
455	White pea beans.....	do.....						23	Immune.
464	Best of all speckled seeded, stringy.	Holland.....	4		15	6			Moderately susceptible.
465	Best of all speckled seeded, stringless.	do.....	6	13	8				Susceptible.
466	Best of all white seeded, stringy.	do.....				8	5		Apparently resistant.
467	Best of all white seeded, stringless.	do.....	25	9					Very susceptible.
468	Dark dun or liver color.....	do.....		23	8				Susceptible.
469	Delicata stringless.....	do.....			6		2	3	Moderately susceptible.
471	Early white farming.....	do.....		9	16		5		Do.
476	Perfect dwarf white stringless.	do.....			13			1	Do.
477	Princess white, stringy.....	do.....		11	5				Susceptible.
479	Transvalian, stringless.....	do.....				38		4	Apparently resistant.
496	Quarantino bianco.....	Italy.....			5	14		7	Somewhat resistant.
525	Osaya Dais Ingen.....	Japan.....			3	4			Apparently somewhat resistant.
528	Suzunari Ingen.....	do.....		29					Susceptible.
548	Short, drab beans with dark ring around the hilum.	Uganda.....	10						Very susceptible.
551	Short, light-green beans with brown ring around the hilum.	do.....						14	Immune.
552	Short, greenish-tan beans with brown ring.	do.....						15	Do.
594	Short, plump Sudan-brown beans.	Urundi.....						28	Do.
597	Purple-chocolate colored marrow beans.	do.....						21	Do.

TABLE III.—Detailed results of greenhouse inoculations with a combined spore suspension of all known biologic forms of *Colletotrichum lindemuthianum* on some of the more resistant varieties and on certain others concerning which the information in Table II is incomplete or lacking—Continued

Variety number	Name or seed characteristics	Source	Number of plants showing the extent of infection indicated						Remarks
			Very severe	Severe	Moderate	Slight	Very slight	None	
603	Finely stippled lavender kidney beans with olive-green stripes.	Urundi.....					3	23	Immune except for small percentage of slightly susceptible sports.
607	Long, finely stippled blue-tan beans with black stripes.do.....						7	Apparently very resistant.
619	Small, lavender mottled beans with dark ring around hilum.do.....						25	Immune.
625	Long, reddish-lavender mottled beans with dark ring around hilum.do.....	18	9					Very susceptible.
631	Small, flat, yellowish-tan mottled beans.do.....			3			13	Immune except for small percentage of susceptible sports.
633	Short, thick purple-chocolate colored beans.do.....						57	Immune.
635	Small, flat pink beansdo.....	6	4					Very susceptible. Resistant except for a fair percentage of susceptible sports.
639	Small, flat dark-tan beans.do.....	2	4	5	8		18	

The reaction of a number of the varieties listed in Table III shows, as expected, that the conditions for infection in the field plot of 1923 were not uniformly favorable enough to reveal the differences in susceptibility brought out by this severe greenhouse test. The same phenomenon has been repeatedly noted by Barrus (1) and others in comparisons of greenhouse and field inoculations with anthracnose. It seems to prove that field conditions are rarely as severe as the artificial greenhouse combination, and that varieties which are seldom more than slightly infected under commercial cultivation may develop the disease to a moderate or even a severe extent in the greenhouse.

The 12 varieties and strains used as controls in this experiment were, with one exception, all severely or very severely infected; the exception (Well's Red Kidney) showed infection only to a moderate extent.

In this test 12 varieties proved to be practically immune to all the 11 cultures or biologic forms of *Colletotrichum lindemuthianum*, e. g., Nos. 115, 413, 455, 551, 552, 594, 597, 603, 607, 619, 631, and 633. However, comparisons of their reaction in earlier greenhouse inoculations and field tests (Table II, columns 6 to 8) show that all but No. 633 are listed in one column or another as very slightly infected. This apparently contradictory evidence is probably due as much to variation in judgment of the observer as to real variation in the extent of infection. While the definitions of immunity (i. e., no infection) and "very slight" infection, in Table I, would seem to

partially eliminate this possibility, yet in actual practice enough intergradation has been observed to make the personal equation an important factor. This, coupled with the fact that the information was obtained in different years and by different people, leads the authors to attach very little significance to expressed differences between "very slight" and "none" as used for this disease.

Coordinating the evidence in both Tables II and III, and considering only those varieties on which there is fairly adequate information with respect to their behavior toward anthracnose and bacterial blight, the resistant material isolated in this study may be classified as follows:

1. Varieties apparently resistant to both anthracnose and bacterial blight:
 - 594, S. P. I. 50850, small brown beans, pole type, from Urundi.
 - 451, S. P. I. 53808, small red kidney, pole type, from Guatemala.
 - 115, S. P. I. 48472-C, large, light-brown striped beans, pole type, from Belgian Congo.
 - 597, S. P. I. 50853, purple-chocolate colored marrow beans, pole type, from Urundi.
 - 118,^a S. P. I. 48472-F, bluish-striped kidney, pole type, from Belgian Congo.
2. Varieties apparently resistant to anthracnose:
 - Infection, none—
 - 551, S. P. I. 52192, dwarf type, from Uganda.
 - 552, S. P. I. 52193, dwarf type, from Uganda.
 - 603, S. P. I. 50859-B, pole type, from Urundi.
 - 619, S. P. I. 50873-A, pole type, from Urundi.
 - 633, S. P. I. 50885, pole type, from Urundi.
 - 638,^a S. P. I. 50888-A, pole type, from Urundi.
 - Infection very slight—
 - 117,^a S. P. I. 48472-E, pole type, from Belgian Congo.
 - 404, S. P. I. 53875, Blanco de vara, pole type, from Guatemala.
 - 413, S. P. I. 53792, De Sarco, pole type, from Guatemala.
 - 455, S. P. I. 53817, white pea beans, pole type, from Guatemala.
 - 607, S. P. I. 50861-C, pole type, from Urundi.
 - 627,^b S. P. I. 50879-B, pole type, from Urundi.
 - Infection slight—
 - 245,^b Beurre blanc nain, dwarf type, from France.
 - 269,^b Flageolet blanc extra, dwarf type, from France.
 - 286, Jaune de la Chine, dwarf type, from France.
 - 299,^b Nain Mangetout extra-hâtif, dwarf type, from France.
 - 301, Nain Parisien, dwarf type, from France.
 - 386, S. P. I. 53784-A, Negro pequeño mata, dwarf type, from Guatemala.
 - 408, 53781, Colorado, pole type, from Guatemala.
 - 424,^a 53775, Negro de vara, pole type, from Guatemala.
 - 466, Best of All, white, stringy, dwarf type, from Holland.
 - 479, Transvalian stringless, dwarf type, from Holland.
 - 525, Osaya Dais Ingen, pole type, from Japan.
 - 617,^b 50872-A, pole type, from Urundi.
 - 618,^b 50872-B, pole type, from Urundi.
 - 631, 50883, pole type, from Urundi.
 - 650,^b 50900-A, pole type, from Urundi.
3. Varieties apparently resistant to bacterial blight:
 - 19, Hodson Wax.
 - 20, Keeney Rustless.
 - 45, French's Horticultural.
 - 72, Robust (Nelson selection).
 - 80, Refugee (Roger selection).
 - 85, White Imperial.
 - 89, Baldwin Wonder Wax, pole.

^a Classified as resistant on basis of negative reaction to the *alpha*, *beta*, and *gamma* biologic forms in greenhouse tests and to all of the 11 forms in the field.

^b Loc. cit.

3. Varieties apparently resistant to bacterial blight—Continued.

- 101, London Horticultural, pole.
- 107, Worcester Mammoth, pole.
- 283, Incomparable, dwarf type, from France.
- 325, Coco bicolore du Pape, pole type, from France.
- 351, Zébré gris, pole type, from France.
- 395, S. P. I. 56584—A, Alubia, white kidney, dwarf type, from Guatemala.
- 454, S. P. I. 56582, Light tannish-pink beans, pole type, from Guatemala.
- 494, Meraviglia di Lione, dwarf type, from Italy.
- 499, Varesotto, dwarf type, from Italy.
- 528, Suzunari Ingen, pole type, from Japan.
- 541, S. P. I. 51079, pole type, from Russia.
- 544, S. P. I. 51082, pole type, from Russia.
- 545, S. P. I. 51083, pole type, from Russia.
- 549, S. P. I. 52191, dwarf type, from Uganda.
- 556, S. P. I. 52198, dwarf type, from Uganda.
- 565, S. P. I. 52201, pole type, from Uganda.
- 577, S. P. I. 50259, pole type, from Urundi.
- 578, S. P. I. 50260, pole type, from Urundi.
- 589, S. P. I. 50846, pole type, from Urundi.
- 615, S. P. I. 50870, pole type, from Urundi.
- 621, S. P. I. 50875, pole type, from Urundi.
- 626, S. P. I. 50879—A, pole type, from Urundi.
- 635, S. P. I. 50886—B, pole type, from Urundi.
- 636, S. P. I. 50886—C, pole type, from Urundi.
- 660, S. P. I. 46370, from Venezuela.
- 661, S. P. I. 46365, from Venezuela.

From the above lists it is seen that, out of a total of 663 varieties and strains tested, 65 are considered more or less resistant to anthracnose or bacterial blight, of which 5 are classified in Group 1, 27 in group 2, and 33 in group 3. Several of the blight-resistant varieties in group 3 which appeared resistant also to anthracnose in the field tests doubtless belong in group 1, but their apparent resistance has not been confirmed by greenhouse inoculation. However, their field behavior confirms the results of Burkholder (3) and others.

Although a number of the varieties, particularly the first six in list 2, are practically immune to anthracnose, no such striking behavior toward bacterial blight was observed. No variety came through the test-plot epidemics of both 1921 and 1923 with less than "slight" blight infection. The degrees of infection less than "slight," defined in Table I, occurred, therefore, mainly on other species of the genus. As Burkholder (3) has pointed out, resistance to blight is manifested in other ways than merely by reduction in number or size of the lesions. The incubation period is noticeably longer and the lesions enlarge more slowly in case of the resistant variety, enabling it to mature a good crop in spite of the disease.

ADAPTABILITY AND DESCRIPTION OF ANTHRACNOSE AND BLIGHT RESISTANT TYPES

The adaptability and commercial value of the few standard American and European varieties, listed in groups 2 and 3, are fairly well known and as shown presently, could probably with advantage supplant certain very susceptible varieties of the same type now being grown in this country. Little is known of the horticultural value of the Central American and East African resistant beans. They are now being increased, selected for uniformity, and studied from the

standpoint of commercial utilization and suitability for hybridizing with certain susceptible American varieties. Although most of them have appeared quite uniform in their resistance, in a few cases much variability in plant characters has been noted. However, the foreign introductions which have thus far shown the least variability in type and which seem the most promising for hybridization are briefly described at this time.

The varieties in group 1 are either very tall pole types or bush beans with the pea-bean habit of growth. The color and shape of seed and extreme lateness make them unsuitable for direct use at least in the northern field-bean sections, and their tough, fibrous, stringy, small pods preclude their use anywhere as snap beans. They have all come fairly true to type and offer perhaps the most promising possibilities for hybridization work.

Of particular interest in group 2 are the S. P. I. Nos. 52192 and 52193 (variety Nos. 551 and 552) which are apparently identical in plant characters and reaction to disease. Both have a dwarf, indeterminate habit, with short, thick, green, tough, pods containing three or four slate green, marrow type seeds. They are much earlier than the varieties in group 1, but are less productive. Both varieties might be used advantageously in crosses with many of our high quality but susceptible garden types, and with the kidney and marrow field beans, to introduce resistance to all the biologic forms of the anthracnose fungus.

The variety *Negro pequeño mata* (No. 386, S. P. I. 53784-A) is perhaps the most promising for introducing anthracnose resistance into white pea beans such as Early Wonder and Michigan Robust. It develops large, spreading, indeterminate plants with dark-green foliage, very late and productive; pods pea-bean type in color and size; seeds black, pea-bean shape and size but somewhat flattened.

The other South American and East African varieties in group 2 are apparently so far removed in horticultural characters from our American types that one would use them in hybridization only as a last resort.

The anthracnose-resistant French varieties in group 2 seem to have not only immediate commercial possibilities, but also offer promising material for crossing with such anthracnose-susceptible American types as Burpee's Stringless Green Pod, Giant Stringless Green Pod, Bountiful, Full Measure, Rogers Improved Green Pod Refugee, Refugee Wax, Sure Crop Wax, Pencil Pod Wax, Burpee Kidney Wax, Kentucky Wonder Pole, etc. Descriptions of these French varieties follow.

No. 245. *Beurre blanc nain*, described by Denaiffe (4, p. 180) and by Robinson (10, p. 82) as grown at Lansing, Mich., is of dwarf, indeterminate growth, about 12 inches high; foliage light green, medium size; flowers white; snap pods stringy, parchmented; of transparent, greenish-yellow color, flat, slightly curved, 5 or 6 seeds per pod, 4 to 5 inches long, $\frac{1}{2}$ to $\frac{5}{8}$ inch wide; season early; dry seed white, medium size, oval in cross section, rounded ends, large pea-bean type. The dried seed is said to be excellent for table use. This variety, because of its resemblance in many ways to a pea-bean type, might be used in crosses with Michigan Robust to secure an anthracnose-resistant pea bean. It would also be a valuable type to use in crosses with American sorts where a white seeded garden or canning variety is desired, but the pods are too pale a yellow to make it useful in hybridizing with a susceptible green podded type to secure a resistant wax-podded segregate.

No. 269. *Flageolet blanc extra*, described by Robinson (10, p. 56) and Denaiffe (4, pp. 142, 143), is said by the former to be the best known and most esteemed

of the tough-podded kidney beans in France. As grown at Lansing, Mich., the plants are of dwarf, indeterminate growth, 12 inches high; foliage small, medium green; flowers white; pods green, oval in cross section, curved, $4\frac{1}{2}$ to 5 inches long, $\frac{3}{8}$ inch wide, stringy, parchmented, 4 or 5 seeds per pod; dry seeds medium size, flat, $\frac{1}{32}$ inch long, $\frac{5}{32}$ inch wide, and $\frac{7}{32}$ inch thick, kidney-shaped, excellent as green shell beans. This variety may be used in hybridization the same as Beurre blanc nain, while the longer, narrower pods make it more desirable for crossing with garden types.

No. 299. *Nain mangetout extra-hâtif*, described by Denaiffe (4, pp. 160, 161). As grown at Lansing, Mich., plants are very dwarf, 6 to 8 inches high, determinate, very early; foliage light green; pods green, round, slightly curved, stringy, parchmented, 3 to $3\frac{1}{2}$ inches long, $\frac{3}{8}$ inch wide, 4 or 5 seeds per pod; dry seed white with light yellow ring around hilum, $\frac{1}{2}$ inch long by $\frac{1}{4}$ inch wide by $\frac{1}{4}$ inch thick, round in cross section, rounded ends. This variety seems to have no direct horticultural uses in this country, but appears of value to use in crosses where a very early, resistant bean is desired.

No. 286. *Jaune de la Chine*, described by Robinson (10, p. 88) and by Denaiffe (4, p. 295). Plants dwarf, 16 inches high, much branched, determinate; foliage medium-sized, light green; pods green, 5 inches long, $\frac{5}{8}$ inch wide, round, slightly curved, stringy, parchmented when old, 5 or 6 seeds per pod; productive, mid-season; seeds yellow, bluish ring around hilum, $\frac{5}{8}$ inch long, ovoid or subspherical in shape. Because of its anthracnose resistance and usefulness as a snap and green shell bean, this variety may be worthy of trial to supplant susceptible green shell types, such as Low's Champion. According to Jarvis (6) *Jaune de la Chine* was formerly much cultivated in this country under the name Yellow Cranberry, but has now almost gone out of use.

No. 301. *Nain Parisien*, described by Robinson (10, p. 59) and by Denaiffe (4, p. 232), has a dwarf plant of vigorous growth, determinate, dark green leaves, lilac-colored flowers; pods straight, cylindrical, 6 to $6\frac{1}{2}$ inches long, dark green with purple streaks that disappear on cooking, parchmented, stringy; very productive, mid season; seeds large, flat, kidney-shaped, streaked dark purple on chamois. The purple streaking on the pods make this variety unsuitable from a market-garden standpoint to supplant susceptible varieties, such as Black Valentine, but the general horticultural characters of *Nain Parisien* make it more useful for hybridizing than the late coarse-vined South American sorts in group 2.

RESISTANCE TO BACTERIAL WILT

The information on bacterial wilt, as explained earlier, is insufficient as a basis for judging relative susceptibility. However, 15 of those varieties inoculated by the "cotyledon method," which appeared least affected and are marked in Table II with the letter c, may on further testing prove to be somewhat resistant to this disease. The results of the needle-prick inoculations on older plants are even less valuable for comparative purposes. Yield of inoculated and control plants would doubtless have given a better basis for comparison than the more or less artificial classification of infection used in these preliminary experiments. Replication of tests and careful comparisons with controls would be necessary, however, to offset possible complicating factors, as natural spread of wilt on controls, infection by other diseases, and differences in time of maturity of the varieties.

The extremely severe type of wilt produced by inoculations in the cotyledon stage is comparable, in the writers' observations, to naturally occurring early season field cases resulting from seed infection, while the symptoms produced by later inoculations are similar to the more prevalent but individually less severe type resulting, apparently, from secondary spread in the field. Occasionally, however, general and severe wilting follows these late infections, as shown by the case illustrated in Plate 3, A.

TESTS ON RELATED SPECIES OF PHASEOLUS AND ON OTHER BEAN GENERA

Lima bean (*Phaseolus lunatus* L.).—Along with the varieties of the common bean there were included, during one or more of the four years of field testing, 21 of the commonly listed American dwarf and pole varieties of lima beans and 62 foreign varieties, from 12 different countries. The short growing season and other unfavorable conditions for this type of bean at the latitude of Lansing, Mich., prevented the obtaining of reliable comparative data. In general, most of the varieties appeared to be more or less susceptible to blight and usually showed a slight to moderate amount of anthracnose and mosaic. More variation was noted with respect to bacterial wilt, the extent of infection ranging from none in the case of Henderson and Wood Prolific, to severe in Burpee Bush from both field and greenhouse inoculations.

Runner beans (*Phaseolus multiflorus* Willd.).—Most of the 57 varieties and strains of runner beans included in the field tests developed both anthracnose and bacterial blight to a slight or moderate extent and frequently showed traces of mosaic. No signs from any of the inoculations with the bacterial wilt organism.

Tepary beans (*Phaseolus acutifolius* Gray.).—A selection of both white and colored seeded varieties tested in field plots showed rarely more than a trace of bacterial blight and a moderate to severe amount of anthracnose. No wilt or mosaic was observed.

Adzuki bean (*Phaseolus angularis* (Willd.) Wright).—Infection slight with bacterial blight and mosaic, and none with anthracnose in field tests; severely infected with bacterial wilt in greenhouse tests.

Mung bean (*Phaseolus aureus* Roxb.).—Traces of bacterial blight in the field; immune to anthracnose but susceptible to wilt in both greenhouse and field tests.

Rice bean (*Phaseolus calcaratus* Roxb.).—No infection from any of the diseases on the two varieties, S. P. I. 46427 and 56072, tested in both field and greenhouse; not inoculated with wilt organism.

Urd bean (*Phaseolus mungo* L.).—No infection from greenhouse inoculations with the *alpha*, *beta*, and *gamma* forms of *Colletotrichum lindemuthianum*. Slightly susceptible to bacterial blight, and moderately susceptible to bacterial wilt in greenhouse tests.

Cowpea (*Vigna sinensis* (L.) Endl.).—Slight to moderate infection in field tests with anthracnose and bacterial blight on majority of 15 varieties from different sources; greenhouse wilt inoculations negative.

Asparagus bean (*Dolichos sesquipedalis*, L.).—Anthracnose occasionally very slight on leaves in greenhouse tests; no positive evidence of bacterial blight and wilt; mosaic usually severe.

Hyacinth bean (*Dolichos lablab* L.).—Dolique Mongette from France, slight mosaic and an undetermined leaf spot resembling bacterial blight.

Jack bean (*Canavalia ensiformis* (L.) DC.).—No signs of any of the four diseases in field tests.

SUMMARY

This paper presents a summary of four years' bean varietal testing for resistance to anthracnose and bacterial blight. The results of preliminary greenhouse and field inoculations with bacterial wilt and notes on the natural occurrence of mosaic, obtained incidental to this study, are also included.

A total of 663 varieties and strains of beans have been tested, of which 170 are American and 493 foreign, the latter being obtained from 23 countries.

The data on relative susceptibility to bacterial blight are limited to field-plot inoculations which, in 1923, were replicated in three different localities. A large proportion of the foreign varieties were also subjected to carefully controlled greenhouse tests with the various biologic forms of the anthracnose fungus. Since some of the varieties were tested in the field only during years unfavorable for epidemic development of one or another of the diseases, the results on these are necessarily of a preliminary nature.

Considering only those varieties on which there is fairly conclusive evidence, a total of 65 appear to possess decided resistance to anthracnose or bacterial blight, of which 5 are resistant to both diseases, 27 to anthracnose alone, and 33 to bacterial blight alone. A few of the blight-resistant types may, however, prove to be also resistant to anthracnose if their apparent resistance in field tests is confirmed by greenhouse inoculations.

Six of the 27 anthracnose-resistant varieties are practically immune to all known biologic forms of the fungus, as shown both by greenhouse and field inoculations. The remainder were affected only to a slight or very slight extent.

No very high degree of resistance to bacterial blight was observed in any variety of *Phaseolus vulgaris* tested. Although additional comparative evidence is required, a number of the foreign varieties, however, appear to be somewhat more resistant than any of our American types.

Very little is thus far known regarding the adaptability of the foreign varieties used in this study for direct commercial cultivation in the United States. Since some of the most resistant are late pole types of tropical or semitropical origin, it is inferred that these, at least, will be mainly valuable for hybridization with our susceptible American types.

Tests conducted on a large collection of varieties of related species and genera of beans are briefly summarized.

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EFFECT OF DEHYDRATION UPON THE BACTERIAL FLORA OF EGGS¹

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INTRODUCTION

The commercial dehydration of broken-out eggs has been developed to minimize the losses incident to shipment and storage where long distances and long periods of time are involved. The reduction in volume and weight alone is significant, but the reduction in the demands on cold-storage facilities and the need for expensive and elaborate packing methods is still more important. Dehydration has made possible the development of large egg-handling establishments in China, which at present supply most of the dried egg used by the baking industry in the United States.

Sound fresh eggs subjected to one of the controlled drying processes make a very desirable dried product. The practices involved in the breaking and mixing of large quantities of commercial eggs, however, introduce so many opportunities for spoilage and contamination that a bacterial examination of the number and character of the bacteria, and a study of the opportunities for the growth of the microorganisms, seem desirable. Bacterial findings must be correlated with the odor, taste, or other evidence of soundness or spoilage in the manufactured product, and with the same properties in the raw materials.

Sources of contamination during dehydration are the hands of the operator, the apparatus, dirt from the air or from the surface of the shell, and the fragments of shell unavoidably left in the product.³ Perhaps the principal source is the occasional single egg which contains large numbers of bacteria but has not developed physical evidence of spoilage by odor or taste. Such eggs have been found and reported in the investigations of Jenkins and Hendrickson.⁴ Also, many eggs which have been damaged in handling become contaminated with microorganisms in considerable numbers before evidence of decomposition is noticeable. Therefore, a mixed product selected as sound by physical examination may at times contain a large number of bacteria per cubic centimeter. It seems fair to assume that a great many bacteria may be introduced without there having been negligence or gross carelessness in the preparation of the original mix.

With such initial bacterial contamination, enormous numbers of bacteria will develop in a comparatively short time, if the mix is

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² This investigation was a part of a bacteriological and chemical study of dried eggs conducted in cooperation with H. W. Redfield, G. C. Swan, and H. I. Macomber. Acknowledgment is due Charles Thom for many suggestions.

³ PENNINGTON, M. E., JENKINSON, M. K., STOCKING, W. A., ROSS, S. H., ST. JOHN, E. Q., HENDRICKSON, N., AND HICKS, W. B. A STUDY OF THE PREPARATION OF FROZEN AND DRIED EGGS IN THE PRODUCING SECTION. U. S. Dept. Agr. Bul. 224, 99 p., illus. 1916.

⁴ JENKINS, M. K., and HENDRICKSON, N. ACCURACY IN COMMERCIAL GRADING OF OPENED EGGS. U. S. Dept. Agr. Bul. 391, 27 p., illus. 1918.

held at a temperature other than that of effective refrigeration. An efficient egg-drying process, therefore, must provide for the immediate chilling or freezing of the broken-out product or for its prompt dehydration.

In the course of dehydration the products are subjected to contact with heated air, which removes, partially at least, the volatile constituents. This removes or changes the odors characteristic of the fresh sound product and reduces or destroys the odors in low-grade eggs incident to spoilage, which would readily be recognized in the liquid egg. The odor of commercially dried eggs, therefore, may be expected to differ sufficiently from that of the fresh product to require careful examination by experienced analysts.

The odor, appearance, and taste of dehydrated egg differ so markedly from those of fresh liquid egg that extensive experience is necessary to coordinate the two kinds of egg.

In planning a bacteriological examination of dehydrated eggs, all of these factors must be taken into consideration. Theoretically, the number of living bacteria in the finished product depends on the number and kind of bacteria in the liquid product, the temperature to which the product is subjected, and the length of time at which that temperature is maintained. Furthermore, in view of the fact that most of the dried eggs used in this country are imported, the effect of the time and conditions of storage upon the bacterial count becomes important, if that count is to be used as an index of quality.

It is believed that the air is an unimportant source of contamination under proper conditions in the factory. This is certainly true of the processes studied in this investigation. Likewise, the amount of contamination from utensils is insignificant, if they are clean and dry. If the utensils are wet or improperly cleaned, however, the product will have more organisms in the portions in contact with such contamination. From the bacteriological standpoint, the moisture content of the dried product, and the evenness of the drying, are the factors which must be closely watched if a desirable and uniform product is to be obtained.

Two dehydration processes for eggs—the spray and vacuum-drum—were studied by the Bureau of Chemistry, United States Department of Agriculture, in the summers of 1922 and 1923. In both of these processes liquid egg was dried to a powder of low moisture content in a short time. Bacterial action during this short drying period was considered negligible. The content of living organisms in the egg powder represents the effect of the temperature, time of exposure at that temperature, and degree of the concentration of the product upon whatever organisms were initially present.

SPRAY PROCESS

GENERAL PROCEDURE

In the spray process, the first method studied, the egg was exposed to a temperature of about 75° C. for 18 to 25 minutes. Briefly, this process consisted in forcing liquid egg, under great pressure, through a fine spray apparatus, into a collecting chamber in which dehydration was accomplished by exposure to a large volume of heated air. The egg particles remained exposed on the floor of the heated chamber for the period of the "run," approximately 20 minutes.

GRADES OF EGGS USED

Two grades of eggs—good (commercial firsts) and inedible—were used. The inedible grade contained white rots, spots, and other types. In view of the fact that the eggs were broken under supervision at the breaking table, it was not thought necessary to candle the good eggs. The inedible eggs were candled and separated into the various classes. Each grade was broken out at the breaking table under the writer's supervision. The liquid egg was thoroughly mixed, and was dried immediately. From the drier, the powder was placed on trays and cooled, and then placed in friction-top cans which were finally sealed with paraffin.

EXPERIMENTS NOS. 1, 2, AND 3

WHOLE EGG.—The eggs used (30-dozen cases for each experiment) were purchased on the open market as first grade eggs. In order to have as uniform a product as possible, the following restrictions were made: All eggs which had a doubtful appearance after being broken out were discarded. If the eggs were "off" in color or odor, they were not used. The few green whites found were discarded, as were eggs in which the yolks were reddened, but not showing development of the embryo. Eggs in any way questionable were discarded.

EXPERIMENTS NOS. 4, 5, 6, AND 7

YOLK.—The quality of the eggs and the grading at the breaking table were the same as those in Experiments Nos. 1, 2, and 3. The yolks were separated carefully from the whites. The yolks from six cases were used in each experiment.

EXPERIMENTS NOS. 8 AND 9

YOLK.—In Experiment No. 8 the yolks of sound eggs, after being mixed, were allowed to stand at the temperature of the breaking room (about 25° C.), for approximately 15 hours before drying, and for approximately 24 hours in Experiment No. 9. Six cases were used in each experiment.

EXPERIMENTS NOS. 10 AND 11

HEATED WHOLE EGG.—Commercial firsts were placed in the sun. The course of their deterioration was followed by candling. The whites were very thin when broken out at the table. Black rots, white rots, and spot eggs were not used. Three cases were used in each experiment.

EXPERIMENT NO. 12

YOLK.—The treatment, grading, and quality of the eggs were the same as those in Experiments Nos. 10 and 11. The yolk membranes were firm, so that no difficulty was encountered in separating. Six cases were used.

EXPERIMENT NO. 13

WHOLE EGG.—The eggs in this and the following experiments were candled. This experiment was made on 28 dozen doubtful eggs and 48 dozen spots. The eggs which were graded as doubtful by the candler had weak yolks and watery whites, and there were some white rots.⁵ All eggs were used except black rots.

⁵ JENKINS, M. K., and HENDRICKSON, N. Op. cit., found that white rots were passed occasionally by candlers.

EXPERIMENT NO. 14

WHOLE EGG.—The egg used consisted of six dozen blood rings, 55 dozen addled eggs, and 15 dozen spots. In the blood rings there were no definitely marked embryos. The addled eggs contained white rots and green whites. All black rots were discarded.

EXPERIMENT NO. 15

WHOLE EGG.—In this experiment 54 dozen addled eggs and 24 dozen mixed rots, but no black rots, were used.

BACTERIOLOGICAL EXAMINATION

Samples of the liquid product were taken for bacteriological examination just before it was taken to the drier. They were collected in sterile glass jars, and placed in the sharp freezer and kept frozen until examined. Samples of the dried product were taken after it was cool, and again just before it was placed in the tin containers.

The odors of the liquid and dried egg were compared carefully. There was no difficulty in determining the quality of the liquid egg, but some volatile material was lost during the drying process, which made grading more difficult in the dried products. The dried product prepared from good eggs could be differentiated very easily from that prepared from rotten eggs. The number of bacteria in the liquid and dried egg is shown in Table I.

TABLE I.—Number of bacteria in liquid and dried egg (spray process)

Sam- ple No. ^a	Type	Quality	Total bacteria on nutrient agar at—		Colon group	BCP lactose agar ^b 37° C. for 2 days	
			37° C. for 2 days	20° C. for 4 days		Total	Acid
1L	Whole	Good	75,000	65,000	100	147,000	8
1D	do	do	450	400	0	500	0
2L	do	do	225,000	225,000	0	380,000	0
2D	do	do	1,700	3,300	0	2,200	900
3L	do	do	7,500	8,000	100	23,000	4,000
3D	do	do	350	350	0	900	600
4L	Yolk	do	530,000	550,000	100,000	630,000	300,000
4D	do	do	550	550	0	700	40
5L	do	do	78,000	80,000	1,000	97,000	1,000
5D	do	do	320	550	0	800	50
6L	do	do	21,000	5,500	0	24,000	1,000
6D	do	do	115	500	0	100	40
7L	do	do	190,000	220,000	100	330,000	0
7D	do	do	1,460	170	10	1,400	0
8L	do	Good, held ^c	350,000	370,000	10,000	600,000	0
8D	do	do	370	285	10	380	60
9L	do	do ^d	650,000	475,000	100,000	3,000,000	1,600,000
9D	do	do	9,100	5,150	10	8,400	0
10L	Whole	Heated	1,700,000	820,000	100,000	2,700,000	300,000
10D	do	do	4,900	1,220	0	6,400	300
11L	do	do	1,600,000	1,050,000	1,000,000	4,400,000	300,000
11D	do	do	1,350	330	10	1,070	0
12L	Yolk	do	500,000	635,000	100,000	1,160,000	500,000
12D	do	do	2,950	650	10	3,500	0
13L	Whole	Doubtful, spots	23,500,000	17,500,000	1,000,000	28,000,000	6,000,000
13D	do	do	58,000	320,000	10	350,000	350,000
14L	do	Blood rings, addled spots.	106,000,000	94,000,000	10,000,000	109,000,000	160,000,000
15L	do	Addled, mixed rots	187,000,000	149,000,000	10,000,000	178,000,000	10,800,000
15D	do	do	410,000	740,000	0	680,000	120,000

^a L designates the liquid product; D designates the dried product.

^b Brom cresol purple lactose agar.

^c Held 15 hours at 25° C.

^d Held 24 hours at 25° C.

All counts were made on the gram basis of the liquid product. For the liquid egg, the sample was weighed in a glass-stoppered flask, on an analytical balance, to the nearest one-hundredth of a gram, and nine times that weight of physiological salt solution (considering that 1 c. c. is equal to 1 gm.) was added. This was called the 1:10 dilution. Sterile glass beads were added to help break up the egg. In the analyses of the dried product, salt solution was added to give approximately the same percentage of total solids as in the 1:10 dilution of the liquid product. This was taken as the 1:10 dilution of the dried product. By this procedure the counts of the dry product were comparable with those of the liquid product.

There was some difficulty in obtaining solutions of the dried product. Apparently the powder did not go completely into solution, but was broken up very finely to form a suspension, so that the product could be regarded as in solution only when no large undissolved particles were present.

The results in Table I show that there is a big reduction in the total number of viable bacteria, and in some cases a complete loss of the colon-aerogenes group, during the drying process.

In addition to the total bacteria counted, a large number of organisms were isolated from the spray-process samples. Some of these organisms of general types, as those of the colon group and a few of the aerobic spore-formers, were identified, but most of them could not be identified absolutely. Organisms of the colon type were predominant in the liquid product, and organisms of the aerogenes type were predominant in the dried products. This change in the types might be explained either by a difference in the heat resistance of the two types, or by a greater sensitiveness of the colon type to the change in pressure involved in the spray process. Further experiments have not been carried out to prove which of the two hypotheses is correct.

VACUUM-DRUM PROCESS

GENERAL PROCEDURE

Experiments in drying eggs by the vacuum-drum method were undertaken in the summer of 1923. Chemical and bacteriological analyses were made on the liquid and dried products, and the odor before and after drying was noted. The plan of the work was similar to that followed in the spray process.

The eggs were broken and dried as in the spray process, and mixed by hand with a perforated disk. Two cases were broken each day. The first case was dried approximately 2 hours after breaking. The second was held in an ice box, and dried approximately 5 hours after breaking. The temperature of the drum was usually about 90° C., but a few of the samples were dried at 88°, and a few at 93°. The exposure was 15 to 20 seconds.

GRADES OF EGGS USED

A few eggs of each grade were candled before the experiments were started. Inspection of the eggs at the breaking table, however, was taken as the best way of ascertaining the quality. Three grades were used—commercial firsts, heated, rots and spots. In general, the commercial firsts proved to be of very good quality. A few showed a little heat. The heated eggs were uniformly of

poorer quality than the first grade. Many of the eggs showed a thinning of the white, and some of the whites were slightly opaque. The rots and spots were classified at the breaking table. Grading was done at the breaking table. Table II gives the results of grading for the eggs used in all the experiments except Nos. 14 and 15. In Experiment No. 14 the numbers and types of eggs determined at the breaking table were 2 black rots, 28 white rots, 2 red rots, 1 moldy, 185 mixed rots, 20 sour, 26 blood rings, and 81 spots. Experiment No. 15 showed 11 red rots, 29 white rots, 187 mixed rots, 24 sour, 13 blood rings, and 88 spots.

One 30-dozen case of eggs was used in each experiment on whole eggs, and two cases in each experiment on whites and yolks.

BACTERIOLOGICAL EXAMINATION

The bacteriological samples were taken at the time of drying in the case of the liquid product and immediately after the drying in the case of the dried product. The method of analysis was the same as that in the experiment with the spray-process dried egg.

Results of the bacteriological examination are shown in Table II.

TABLE II.—Number of bacteria in liquid and dried eggs (vacuum-drum process)

Sam- ple No. ^a	Type	Quality	Total bacteria on nutrient agar at—		Colon group	BCP lactose agar ^b 37° C. for 2 days	
			37° C. for 2 days	20° C. for 4 days		Total	Acid
1L	Whole	Good	54,000	137,000	10,000		
1D	do	do	45,000	67,000	10		
2L	White	do	2,600	2,850	1,000		
2D	do	do	40	60	0		
3L	do	do	166,000	189,000	10,000	111,000	53,000
3D	do	do	18,000	21,000	1,000	18,000	8,300
4L	Yolk	do	2,950	1,600	100		
4D	do	do	1,800	900	100		
5L	do	do	248,000	235,000	10,000	210,000	10,000
5D	do	do	22,000	30,000	0	19,000	61,000
6L	White	Good, held ^c	207,000,000	166,000,000	10,000		
6D	do	do	30,000	40,000	10		
7L	do	do ^c	4,400,000	4,900,000	1,000,000	5,400,000	100,000
7D	do	do	210,000	100,000	10	260,000	9,000
8L	Yolk	do ^c	600,000,000	600,000,000	100,000		
8D	do	do	1,800,000	900,000	1,000		
9L	do	do ^d	1,010,000,000	850,000,000	100,000,000	640,000,000	280,000,000
9D	do	do	22,400,000	35,000,000	10,000	16,000,000	12,000,000
10L	Whole	Heated	15,400,000	15,400,000	100,000	13,300,000	290,000
10D	do	do	760,000	6,400,000	10	750,000	670,000
11L	do	do	7,500,000	7,600,000	1,000,000	6,900,000	800,000
11D	do	do	120,000	180,000	0	200,000	100,000
12L	White	do	4,500,000	1,500,000	100,000	6,500,000	600,000
12D	do	do	1,010,000	1,620,000	10,000	740,000	40,000
13L	Yolk	do	10,000,000	17,000,000	1,000,000	4,200,000	3,100,000
13D	do	do	140,000	260,000	10,000	50,000	40,000
14L	Whole	Rots	164,000,000	170,000,000	10,000,000	100,000,000	33,000,000
14D	do	do	2,400,000	3,100,000	10,000	1,200,000	600,000
15L	do	do	108,000,000	150,000,000	10,000,000	104,000,000	25,000,000
15D	do	do	120,000	170,000	100	150,000	100,000

^a L designates the liquid product; D designates the dried product.
^b Brom cresol purple lactose agar.
^c Held 24 hours before drying.
^d Held 30 hours before drying.

The eggs dried by the vacuum process showed a smaller percentage decrease in the total count than the eggs dried by the spray process. This is explained by the fact that the vacuum process was less effective in killing the nonsporulating organisms.

RESULTS OF BACTERIOLOGICAL EXAMINATION

The heat applied to eggs by both processes killed a certain percentage of the organisms. This percentage increased as the total number increased, and varied with the type of organism. In most of the experiments the decrease in the numbers of the colon group was very large; the decrease in the numbers of the total lactose fermenters, as determined by the plate method, was decidedly smaller. A comparison of the counts on eggs dried by both methods showed that the vacuum-drum process is not as efficient as the spray process in killing bacteria.

The odor of the dried product was much less pronounced than that of the liquid product. Furthermore, the odor was stronger while the product was warm from the drier than after it had been cooled. Counts of the viable bacteria furnish little basis for estimating the quality of such products, especially where the details of their histories are not known.

EFFECT OF STORAGE

In order to determine the effect of temperature and time on the bacterial count, samples of the various grades of dried egg made by the spray process were stored for long periods.

The samples were placed in glass-stoppered salt-mouth bottles, sealed with paraffin, and held at 20° C., at room temperature (approximately 25°) and at 37°. By sealing with paraffin, the factor of variable humidity was eliminated. Total counts on plain agar incubated at 20° and 37°, total counts and the number of acid formers on brom cresol purple lactose agar, and counts of the colon group, were made at the beginning, at the end of 3 months, and at the end of 10 months. The results are shown in Tables III, IV, and V.

TABLE III.—*Effect of storage on bacterial counts of dehydrated eggs*
[Samples held in sealed bottles at approximately 25° C. and examined at the dates indicated] ^a

Sam- ple No.	Type	Quality	Bacteria on plain agar held at—		Colon group	Bacteria on BCP agar ^b held at 20° C. for 4 days	
			20° C. for 4 days	37° C. for 2 days		Total	Acid
	June 20, 1922 (start):						
1	Whole	Good	350	350	0	900	600
2	Yolk	do	550	550	0	700	40
3	Whole	Heated	4,900	1,220	0	6,400	300
4	Yolk	do	2,950	650	10	3,500	0
5	Whole	Rotten	58,000	320,000	10	350,000	350,000
6	Do	do	410,000	740,000	0	680,000	120,000
	October 31, 1922:						
1	Whole	Good	230	145	0	200	0
2	Yolk	do	350	135	0	470	0
3	Whole	Heated	1,900	300	0	2,400	0
4	Yolk	do	2,800	95	0	1,900	0
5	Whole	Rotten	55,000	47,000	0	51,000	0
6	Do	do	145,000	101,000	0	87,000	0
	May 7, 1923 (37° C. for 2 days):						
1	Whole	Good	320	260	0	170	50
2	Yolk	do	335	170	0	290	40
3	Whole	Heated	930	210	0	210	30
4	Yolk	do	1,250	135	0	120	10
5	Whole	Rotten	36,000	26,000	0	29,000	27,000
6	Do	do	30,000	20,000	0	23,000	15,000

^a The samples used in this experiment were dried by the spray process.
^b Brown cresol purple lactose agar.

TABLE IV.—*Effect of storage on bacterial counts of dehydrated eggs*

[Samples held in sealed bottles at 20° C., and examined at the dates indicated] ^a

Sam- ple No.	Type	Quality	Bacteria on plain agar held at—		Colon group	Bacteria on BCP ^b agar held at 20° C. for 4 days	
			20° C. for 4 days	37° C. for 2 days		Total	Acid
	July 11, 1922 (start):						
1	Whole.....	Good.....	350	175	0	370	30
2	Yolk.....	do.....	550	235	0	530	50
3	Whole.....	Heated.....	2,900	225	100	3,400	900
4	Yolk.....	do.....	1,025	75	0	1,130	0
5	Whole.....	Rotten.....	250,000	177,000	0	280,000	170,000
6	Do.....	do.....	235,000	385,000	0	970,000	200,000
	October 30, 1922:						
1	Whole.....	Good.....	355	240	0	280	0
2	Yolk.....	do.....	530	325	0	470	0
3	Whole.....	Heated.....	4,250	480	0	3,200	0
4	Yolk.....	do.....	9,350	175	0	2,300	0
5	Whole.....	Rotten.....	95,000	93,000	0	86,000	0
6	Do.....	do.....	430,000	350,000	0	235,000	0
	May 7, 1923 (37° C. for 2 days):						
1	Whole.....	Good.....	300	235	0	210	30
2	Yolk.....	do.....	490	245	0	150	0
3	Whole.....	Heated.....	1,400	700	0	440	220
4	Yolk.....	do.....	780	100	0	40	0
5	Whole.....	Rotten.....	41,000	42,000	0	32,000	11,000
6	Do.....	do.....	146,000	118,000	0	108,000	78,000

^a The samples used in this experiment were dried by the spray process.

^b Brom cresol purple lactose agar.

TABLE V.—*Effect of storage on bacterial counts of dehydrated eggs*

[Samples held in sealed bottles at 37° C., and examined at the dates indicated] ^a

Sample No.	Type	Quality	Bacteria on plain agar held at—		Colon group	Bacteria on BCP ^b agar held at 20° C. for 4 days	
			20° C. for 4 days	37° C. for 2 days		Total	Acid
	July 11, 1922 (start):						
1	Whole	Good	350	175	0	370	30
2	Yolk	do	550	235	0	530	50
3	Whole	Heated	2,900	225	100	3,400	900
4	Yolk	do	1,025	75	0	1,130	0
5	Whole	Rotten	250,000	177,000	0	280,000	170,000
6	Do	do	235,000	385,000	0	970,000	200,000
	October 31, 1922:						
1	Whole	Good	235	215	0	190	0
2	Yolk	do	610	230	0	470	0
3	Whole	Heated	2,300	130	0	1,600	0
4	Yolk	do	540	65	0	430	0
5	Whole	Rotten	7,500	7,500	0	6,000	0
6	Do	do	81,000	86,000	0	70,000	0
	May 7, 1923 (37° C. for 2 days):						
1	Whole	Good	300	115	0	80	20
2	Yolk	do	530	100	0	150	0
3	Whole	Heated	335	35	0	60	30
4	Yolk	do	130	20	0	0	0
5	Whole	Rotten	600	1,250	0	700	0
6	Do	do	1,350	1,300	0	1,400	300

^a The samples used in this experiment were dried by the spray process.

^b Brom cresol purple lactose agar.

The results reported in Tables III, IV, and V show that the number of viable organisms generally decreases as a result of storage. The extent of this decrease was studied in relation to the quality of the egg, the length of time in storage, and the temperature of storage. Good egg shows a much smaller decrease than poor material. Egg stored at a high temperature, or for a long time, loses a larger percentage of viable organisms than egg stored at a lower temperature or for a shorter time.

There was no visible deterioration in the product, but a distinct odor suggesting rancidity developed in all samples. This odor was most pronounced in the product made from low-grade eggs and held at the highest temperature.

The total colony count of the product made from good eggs remained practically stationary over a period of 10 months. The reason for this result is evident when the type of surviving organism is considered. The greater number of organisms which survived the heating process were spore formers. Furthermore, the spores did not germinate during the storage period, probably because the water content was too low, but they did not die during that period. Consequently, the count was an enumeration of the viable spores at each examination. In the samples of breaking stock having a high total count, however, many more of the nonsporulating organisms survived the heating process. These samples, therefore, showed a reduction in the total bacterial count, as these forms died during storage.

The effect of storage on the specific counts was marked. Samples which showed organisms of the colon group at the time of drying did not show them at the end of three months. All these results on the colon group were based on 1 c. c. of the 1:10 dilution. The same results were obtained with the acid formers. These also were absent in 1 c. c. of the 1:10 dilutions at the end of three months. At the end of 10 months, counts of the acid formers were made on plates held at 37° C. instead of 20°. Evidently the change in the temperature of incubation was all that was necessary for a different group of lactose fermenters to develop. These figures, therefore, lose any comparative significance with the preceding determination.

CONCLUSIONS

The count of viable bacteria in freshly prepared dehydrated egg varies, in general, with the quality of the raw product and the method of dehydration. The counts in the product prepared from whole eggs by the spray process varied from 350 in the good egg to 1,160,000 in the spots. In the product prepared by the vacuum-drum process from whole egg the counts varied from 45,000 in the good egg to 2,400,000 in the rots. In general, the yolk showed a higher number than the whites from the same whole egg.

The plate count of spray-process dehydrated egg held in storage depends on the initial count, the length of time in storage, and the temperature of storage. An initial count of 350 in good egg decreased to 300 in 10 months when held at 37° C. and at 20°, while a count of 235,000 in rotten eggs decreased to 1,350 when held at 37°. In one sample of the rotten eggs held at 20° the count increased from

235,000 to 430,000 in three months. A second sample showed a decrease from 250,000 to 95,000 at the end of three months and a further decrease to 41,000 at the end of 10 months.

The odor which is characteristic of poor-quality eggs is lost to some extent during the dehydration process.

An odor similar to rancidity develops in egg powder held at the various temperatures. This odor was most pronounced in rotten eggs held at 37° C.

THE EFFECT OF LOW TEMPERATURES ON *BRUCHUS OBTECTUS* SAY, AN INSECT AFFECTING SEED¹

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INTRODUCTION

The common bean weevil, *Bruchus obtectus* Say, has been the subject of considerable study. This is probably due to its economic importance and the fact that it is a fairly simple insect with which to work. It can be readily propagated in large numbers, ordinary laboratory conditions constitute a normal habitat, and there is freedom from complications due to periodicity in its life cycle.

The investigation presented here covers two phases. The first is concerned with the general phenomenon of supercooling and freezing, as found in *Bruchus obtectus*. Working along the lines suggested by Bachmetjew (3)², attention has been given to the individual freezing of larvae, pupae, and adults. The second phase deals with the control of this insect by means of low temperatures. It is generally known that temperatures such as obtain in midwinter in the Northern States are fatal to *Bruchus obtectus*. The upper limits of these temperatures have not previously been worked out, although data on the effect of continued cold storage on the bean weevil have been recently published (10).

REVIEW OF LITERATURE

Much of the earlier work on the freezing of living tissues was done by botanists. An admirable résumé and bibliography of that work, together with a summary of the latest work on freezing of plant tissues, is given by Rosa (14). The outstanding worker on the subject of vital temperature in insects is Bachmetjew (1, 2). His first volume contains a review of the theories contributed up to his time, and then extensive data on supercooling and freezing considered from the standpoint of the following influences: Rate at which the insect was cooled, sex and development, hunger, repeating the act of cooling the lymph, lymph coefficient, and season. In an appendix at the end of his first volume is a figure of the apparatus used. His second volume was published later (1907) and gives but little attention to the subject of vital temperature.

Pirsch (13) reviews some of the earlier work on individual freezing of insect larvae, particularly with reference to bees. His citation includes references to the earliest users of the electrothermal method of taking insect temperatures.

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² Reference is made by number (italic) to "Literature cited," p. 181.

Hine (7) reports observations on the effect of freezing insect larvae. Those of *Bellura obliqua* were subjected to temperatures below 0° F. and revived. He records that larvae were frozen solid and could be snapped in two, but the pieces were alive when thawed out. He also records observations on tomato hawk moth larvae. Those that had pupuated before the first frosts came carried through normally; immature forms were frozen as the plant was frozen, those on the tips being killed with the first touch of frost while those on the lower parts of the plant were not killed until frost struck that part of the plant. Sir John Ross's account of his Arctic expedition is quoted by Hine. Ross records that *Larria rossi* withstood successive freezings of -40° F. Further references along the same lines are found in Houlbert (8, p. 255). This writer, quoting Justi, a worker in 1753, reports that larvae could be frozen so hard that they could be snapped in two like ice. If the frozen larvae were permitted to return to normal temperatures, then development proceeded normally.

Duval and Portier (4) found the temperature limits of *Cossus cossus* to be -22° C. They report larvae so hard as to be easily broken in two without water appearing at the point of section. They incline to accept Bachmetjew's conclusion that only the intercellular water freezes and the rest supercools.

The latest paper available to the writer is that of Knight (9) on the nature of the color pattern of *Perillus bioculatus* Fabr., which contains a table of data on the supercooling of that insect considered from the standpoints of season (involving the colloidal state of the lymph) and repetitions of freezing.

EXPERIMENTAL WORK ON SUPERCOOLING AND FREEZING

METHOD

The electrothermal method of taking individual temperatures was used. This method is described by Taylor (17), Harvey (6), and White (18). A portion of the apparatus is figured by Pirsch (13), but in the present study a mirror galvanometer was substituted. This provided an admirable indicator of high sensitivity. A modification of the method for taking individual temperatures will be described later.

In each case of individual freezing tabulated in Table I, a finely pointed thermocouple of constantan and copper was thrust into the insect at approximately the ventral middle. The temperature readings were taken at intervals while the insect was being cooled.

It was found best to avoid using thermocouples made of heavy wire, the best and most uniform results being obtained by the use of No. 36 wire. The only trouble encountered with this wire was short-circuiting due to the rapid wearing of the silk insulation. Erratic jumping of the galvanometer soon gave notice of this trouble. When heavy wire was used, even though it could be tapered to a very fine point by means of acid, considerable difference was found to persist between the temperature registered by the instrument and a control thermometer.

All the specimens used for individual freezing were taken from exactly the same environmental conditions, so that variables due to changes in environment were eliminated. The variables due to

stage (and therefore also probable differences in the colloidal character of the lymph) have been considered, as well as the influence of the rate at which the insect was cooled.

GENERAL DISCUSSION

The typical graph of an insect's reaction to temperature, both high and low, is given in great detail in Bachmetjew's second volume (2). Knight (10) gives a simplified graph, which includes the essential features with which the writer is concerned.

There is first a gradual lowering of the insect's temperature to a point designated by Bachmetjew as the "critical point." It is presumed that the freezing of the body fluids causes the typical rebound to a point which the same author calls the "freezing point of the lymph." Bachmetjew concluded that it was necessary to again cool the insect to its supercooling temperature, or "critical point," after the rebound had taken place, before death ensued. It has been the experience of the writer that one rebound was sufficient to cause death. This was also the experience of Pirsch (13) and Knight (9). Careful examination of the data on which Bachmetjew based his conclusions shows considerable discrepancy. If the freezing was hurried or incomplete it is possible that muscular contractions after the insect thawed out again might have given the impression that life still remained. The data presented in this paper show that there is also a distinct ante-mortem zone of muscular activity. From the purely physical standpoint it seems reasonable to conclude that crystallization results in a disruption of cell tissue which, while not necessarily fatal to a plant, is fatal to an insect. There is a possibility, however, that when a fairly large insect is frozen the disruption of cell tissue may be only local and not sufficient to cause death. This would be most likely in the case of an insect which has been impaled on a thermocouple point.

FREEZING EXPERIMENTS WITH BRUCHUS OBTECTUS

Table I gives the results obtained by freezing 25 individuals in each stage—adults, pupae, and larvae. The adults were taken out of the bean just previous to emergence. This stage was the most satisfactory from the experimental standpoint, since variables due to the more active metabolism, histolysis, and histogenesis, encountered in the more immature stages, were absent. The criterion of color was used to determine approximately the age of the pupae; the age of the larvae was determined by size.

TABLE I.—Data on freezing of *Bruchus obtectus*

UNEMERGED ADULTS

Temperature at start	Time taken to reach 0° C.	Time taken to reach super-cooling point	Rate at which insect was cooled	Super-cooling point	Re-bound point	Remarks
° C.	Seconds	Seconds	Degrees per minute	° C.	° C.	
2	3	18	0.63	-9.50	-7.75	
18	19	35	.55	-8.75	-5.80	
15	19	35	.56	-9.00	-7.20	
5	8	29	.40	-8.40	-5.85	
7.5	14	36	.45	-10.00	-6.50	
5	7	32	.36	-9.00	-6.75	
0	-----	23	.41	-9.50	-5.00	
17	26	50	.46	-11.00	-7.25	
14	20	43	.35	-8.00	-4.25	
15	28	47	.39	-7.50	-3.75	
8	13	34	.37	-7.75	-4.30	
3	15	40	.32	-8.00	-4.75	
10	14	35	.36	-7.50	-3.75	
15	14	40	.44	-11.50	-8.50	
13	15	36	.41	-8.60	-4.80	
9	7	25	.47	-8.50	-3.75	
12	-----	38	-----	-7.75	-5.30	
8	6	19	.58	-7.60	-4.50	
10	13	38	.29	-7.75	-4.25	
1	2	6	1.81	-7.25	-6.50	
13	18	43	.38	-9.50	-5.75	
5	14	33	.38	-7.25	-4.00	
10	12	28	.45	-7.25	-3.50	
12	-----	16	-----	-8.75	-7.25	
9.5	9	32	.50	-11.50	-7.75	
Average	-----	-----	-----	-8.68	-5.55	
PUPAE						
15	24	61	0.26	-9.50	-5.00	Cream-colored.
10	12	45	.29	-9.80	-5.25	Light brown; wings grayed.
8	11	33	.43	-9.50	-4.50	Do.
9	11	29	.46	-8.25	-3.75	Cream-colored.
15	15	40	.44	-11.00	-5.75	Do.
8	9	40	.38	-11.75	-6.00	Do.
10	14	36	.36	-8.00	-3.75	Cream-colored; wings grayed.
8	13	35	.36	-8.00	-4.00	Cream-colored.
10	15	37	.43	-9.50	-4.25	Do.
5	3	25	.44	-9.75	-5.00	Do.
0	-----	55	.23	-12.50	-6.25	Do.
-6	-----	23	.14	-9.25	-5.00	Red-brown; wings black.
12	17	45	.34	-9.60	-5.80	Cream-colored.
5	6	21	.52	-7.75	-4.80	Red-brown; wings grayed.
0	-----	28	.35	-9.70	-5.20	Cream-colored.
14	12	40	.32	-9.00	-5.75	Do.
8	18	40	.29	-6.50	-4.25	Red-brown; wings grayed.
10	25	57	.23	-7.25	-4.50	Cream-colored.
16	18	40	.39	-8.50	-5.25	Thorax black; wings black.
11	15	45	.33	-9.75	-6.75	Red-brown; wings gray.
16	20	40	.34	-6.75	-3.80	Cream-colored.
9	15	35	.35	-7.00	-4.50	Do.
-5	-----	15	.43	-11.50	-5.25	Do.
5	9	26	.53	-9.00	-6.75	Do.
0	-----	23	.50	-11.50	-7.00	Do.
Average	-----	-----	-----	-9.22	-5.12	

TABLE I.—Data on freezing of *Bruchus obtectus*—Continued

LARVAE

Tem- pera- ture at start	Time taken to reach 0° C.	Time taken to reach super- cooling point	Rate at which insect was cooled	Super- cooling point	Re- bound point	Remarks
° C.	Seconds	Seconds	Degrees per minute	° C.	° C.	
15	19	48	0.35	-10.20	-7.20	Less than quarter grown.
15	20	48	.34	-9.50	-5.50	Full grown.
-2.5	-----	24	.36	-8.75	-5.50	Half grown.
3	8	39	.28	-8.80	-5.25	Full grown.
1	2	27	.35	-8.75	-6.60	Do.
15	30	62	.25	-8.00	-4.00	Two-thirds grown.
-3	-----	33	.28	-9.20	-5.10	Half grown.
9	11	31	.42	-8.40	-4.40	Full grown.
12	24	54	.28	-8.30	-6.75	Quarter grown.
10	14	60	.29	-13.25	-9.75	One-third grown.
7	10	52	.29	-12.50	-11.00	Quarter grown.
11	12	32	.50	-10.00	-7.50	Two-thirds grown.
-3	-----	14	.66	-9.30	-5.25	Do.
15	23	50	.32	-8.75	-6.10	One-third grown.
7	11	31	.45	-9.00	-6.50	Full grown.
14	15	48	.30	-10.00	-7.25	Do.
19	39	70	.40	-12.50	-8.80	Do.
3	2	8	1.96	-11.75	-8.60	Quarter grown.
16	20	70	.24	-12.00	-6.10	Full grown.
19	9	26	.66	-11.25	-7.75	Do.
10	4	9	2.00	-10.00	-5.00	Two-thirds grown.
-----	3	5	1.92	-9.60	-4.50	Half grown.
18	5	10	2.05	-10.25	-7.75	Do.
19	5	10	1.80	-9.00	-6.00	Do.
-----	3	7	2.31	-9.25	-4.75	One-third grown.
Average	-----	-----	-----	-9.53	-6.52	

The averages in Table I are brought together here for convenience of reference:

	Adults	Pupae	Larvae
Supercooling point.....	° C -8.68	° C -9.22	° C -9.53
Rebound point.....	-5.55	-5.12	-6.52

This shows a progressive lowering of the supercooling point from adults to larvae. The differences between these are slight, and may find their explanation in the metamorphic differences existing between these three stages. It is interesting to note that the supercooling point is depressed in the purely vegetative larvae and is highest in the adult. It should be noted, however, that in the case of the unemerged adults much greater uniformity of development is found than in any of the more immature stages. These first named were all obtainable at a point just previous to emergence. The pupae on the other hand, presented variations due to differences in the progress of histolysis and histogenesis, while the larvae were in all stages of growth, from very small specimens just big enough to contain the thermocouple point to full-grown larvae full of fat body.

Reference to Figure 1, B, will show that while the general trend of the curve is similar to those of Figure 2, A and C, the points are more widely separated. This suggests that individual differences among pupae are due to differences in *kind* while those of the larvae are dif-

ferences in *degree*. Stated in another way, this means that the larval period is one in which accumulation of material of the same kind and with the same properties goes on from the first establishing of the larva within the bean to its final maturity as a larva, while with the pupa histolysis and histogenesis produce changes in the chemical and physical constituents of the body contents, these changes varying with the age of the pupa. With the advent of the adult these changes are completed and the short period of quiescence before emergence simply permits of a hardening process taking place whereby the chitin becomes hard and brittle.

EFFECT OF INJURY ON THE INSECT'S RESISTANCE

Early in the course of the work the question as to the effect of injury on the resistance of these insects was recognized. Practically all the work of previous authors has been done with the thermocouple method which involves piercing the insect. Is the vital temperature so recorded any index of the capacity of the insect to resist low temperature? In order to test this, quiescent adults, pupae, and larvae

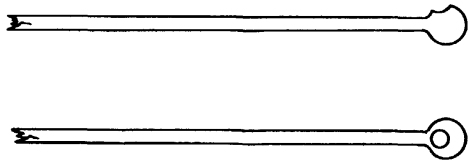


FIG. 1.—Diagram of glass tube used in freezing insects without injury due to piercing by thermocouple

were removed from the beans and put into a gelatin capsule through which the thermocouple point was thrust. The specimens were then uninjured and were lying around the couple point. The method was then modified in order to secure more rigidity by taking a piece of glass tubing, blowing a bulb at the end and then making a small hole in the bulb. The thermocouple was then thrust down until it was almost to the bottom of the bulb and the insects placed down in the bulb and close to the couple point. When several insects are so placed it so happens that one of them will "rebound," but the heat given off will be dispersed and only a mere indication of the rebound will be recorded by the instrument. Rebound under these circumstances then is simply an indication that inoculation has taken place and the amount is not significant (Table II).

TABLE II.—Experiments to determine the effect of injury on an insect's resistance

Super-cooling point	Rebound point	Stage	Number used	Result
° C.	° C.			
-13. 60	-10. 70	Pupal...	14	All died.
-17. 00	None.	...do....	15	Lived.
-20. 00	None.	...do....	3	Do.
-20. 00	None.	...do....	3	Do.
-20. 00	None.	...do....	3	Do.
-13. 25	None.	Larval ..	1	Do.
-16. 50	None.	...do....	1	Do.
-16. 00	None.	...do....	1	Do.
-20. 00	None.	...do....	24	Do.
-17. 00	Slight.	...do....	4	Three lived.*
-15. 50	None.	...do....	1	Lived.
-17. 00	None.	Adult ^b ...	10	Seven lived.
-17. 00	None.	...do. ^b ...	15	Six lived.
-22. 00	None.	...do. ^b ...	10	One lived.

* Of these four larvae one was slightly injured putting it into the tube and a thin film of its body fluid held it to the glass. The couple was not touching it. Only that larva died; the other three lived.
^b Unemerged adults.

Comparing these figures (Table II) with the averages for individual freezings quoted above, it becomes apparent that all these stages are capable of resisting lower temperatures when they are not injured. This may be due to the cuticula preventing the rebound or, in other words, preventing "inoculation." It may be that the presence of free water in the vicinity of the couple point may hurry inoculation in the case of the injured specimens. That an uninjured organism can better withstand extreme conditions than an injured one is a point that has been overlooked in much previous work.

When an insect is impaled on a thermocouple point and cooled, the temperature recorded is that of the lymph and broken-down tissue in the immediate vicinity of the point. It may be of some physiological significance that the freezing point of the impaled insect's lymph is not the same as that of the insect as a living uninjured organism.

The experiments described in the second part of this paper, when bean weevils in all stages were able to withstand temperatures as low as those shown to be the limit for injured specimens and for a greater length of time, seems to establish this point still further.

RELATION BETWEEN SUPERCOOLING AND REBOUND

Figure 2, A, B, and C, are dot charts wherein the supercooling temperatures have been plotted against the rebound points for each set of data on individual freezing. The charts suggest that there is a relationship between the two factors, so the Pearsonian method of arriving at the coefficient of correlation was used (12).

The correlations that exist between supercooling and rebound are thus found to be:

	Correlation	Probable error
Adults.....	+0.779	0.053
Pupae.....	+ .614	.084
Larvae.....	+ .890	.028

These high correlations indicate very definitely that the supercooling point bears a distinct relationship to the rebound point. Just what this relationship is, however, is another matter. According to chemical law (11, pp. 172-180) the rebound point should be the same no matter how low supercooling goes. If this law applies, then the explanation of this correlation must be sought elsewhere.

Knight (9) showed that when freezing *P. bioculatus* Fabr. a thermocouple placed near the insect registered a rise in temperature when the insect rebounded, showing that heat was given off to the surrounding air. This means, of course, that the thermocouple on which the insect is impaled does not register the total amount of heat of crystallization given off by the insect but that some is lost by radiation. These insects (*B. obtectus*) are very small, not more than 3 or 4 mm. long, so that their surface is very great in proportion to their mass. It follows then, that radiation to the air of the chamber must consume a considerable portion of the heat of crystallization.

Because radiation varies as the difference in temperature between the source of the heat and the surrounding medium, it is reasonable

to assume that the lower the temperature of the cooling chamber the more heat will be absorbed by radiation when the insect rebounds. The lower the insect supercools the lower will be the point to which the heat of crystallization available to the thermocouple will enable the temperature to rise, or, in other words, the lower will be the point of rebound. If this is true the correlation between supercooling and rebound points is simply due to the amounts of radiation being related to the temperature of the cooling chamber. Whatever the explanation may be, the fact of the existence of this correlation seems to have been demonstrated.

RELATION BETWEEN SUPERCOOLING AND THE RATE AT WHICH THE INSECT WAS COOLED

Bachmetjew expressed the belief that possibly there may be a relationship between the rate at which the insect is cooled and the supercooling point. His "abkühlungsgeschwindigkeit" is described as a "shadow in the background."

Figure 3, A, B, and C, are dot charts wherein the supercooling points have been plotted against the rate at which the insect was cooled. In computing these rates the time taken to bring the insect from 0° C. to the supercooling point was used in order that the rates could all be calculated from a common starting point, as the initial temperature of the bath varied. The coefficients of correlation and the probable errors for the three stages are as follows:

	Coefficients	Probable errors
Adults.....	-0.095	0.138
Pupae.....	- .190	.129
Larvae.....	+ .015	.134

It is thus seen that the rate of supercooling has no part in establishing the temperature of supercooling; the coefficient is less in each case than the figure required to establish even a bare relationship. It might be added here that the data for larvae offer the best opportunity for verifying this phase of the question. Previous to obtaining the latter half of these data the suction of an ordinary tap pump was used to cool the ether, and differences in rate were slight. Later, a suction pump was obtained which reduced temperature very rapidly, so that there is a much greater variation in the rates.

EXPERIMENTS ON THE UPPER LIMITS OF LOW TEMPERATURES FATAL TO BRUCHUS OBTECTUS SAY

HISTORICAL

Garman (5) subjected weevils to outside temperatures. These are presumably mean temperatures for the periods stated. His general conclusion was that a temperature of about 0° F. for a period of 24 hours was a complete control. According to the data of this paper the eggs are most resistant and the larvae least resistant. This writer suggests that the reason for the freedom of Canadian-grown seed from infestation is due to the inability of the weevils to survive the rigor of the winter. Sanderson (15, 16) discusses this subject in some detail for insects generally.

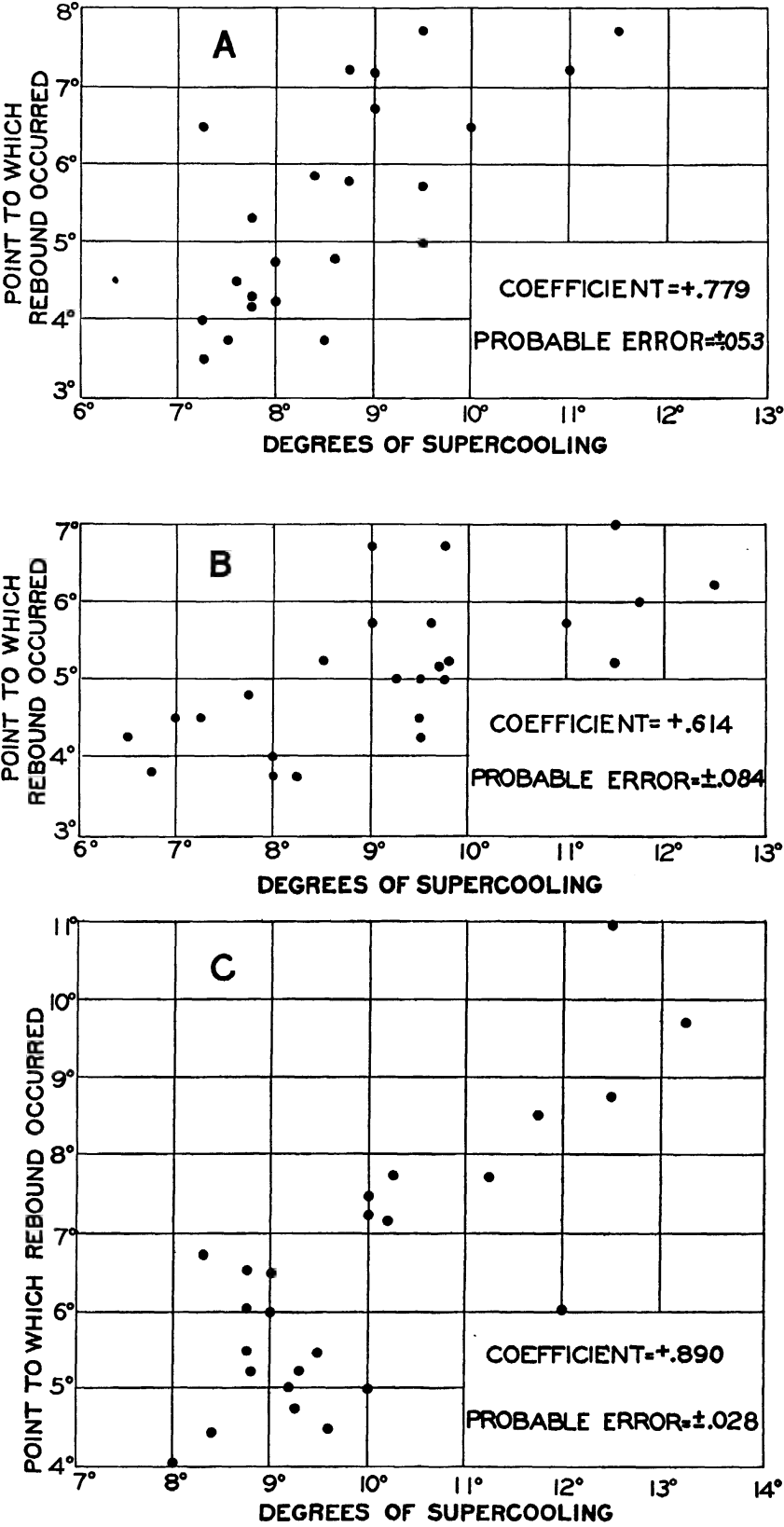


FIG. 2.—Relation between supercooling and rebound. A, adults; B, pupae; C, eggs

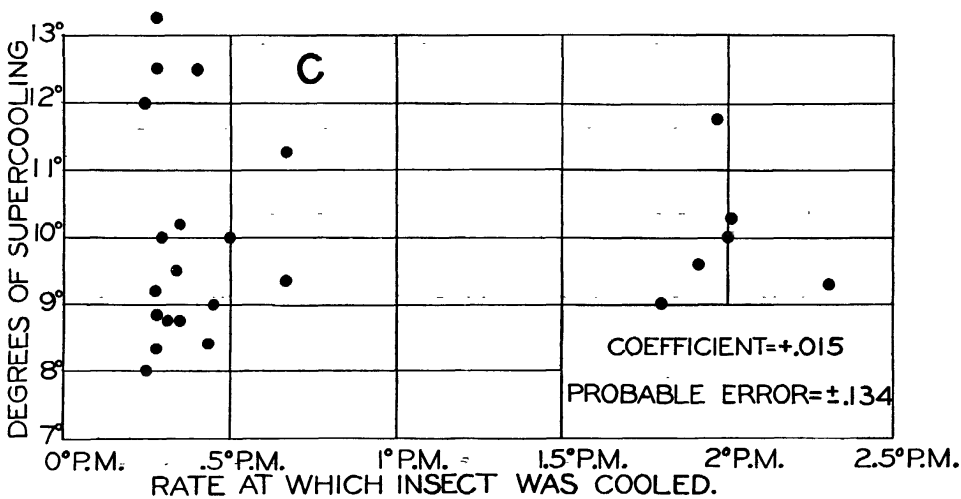
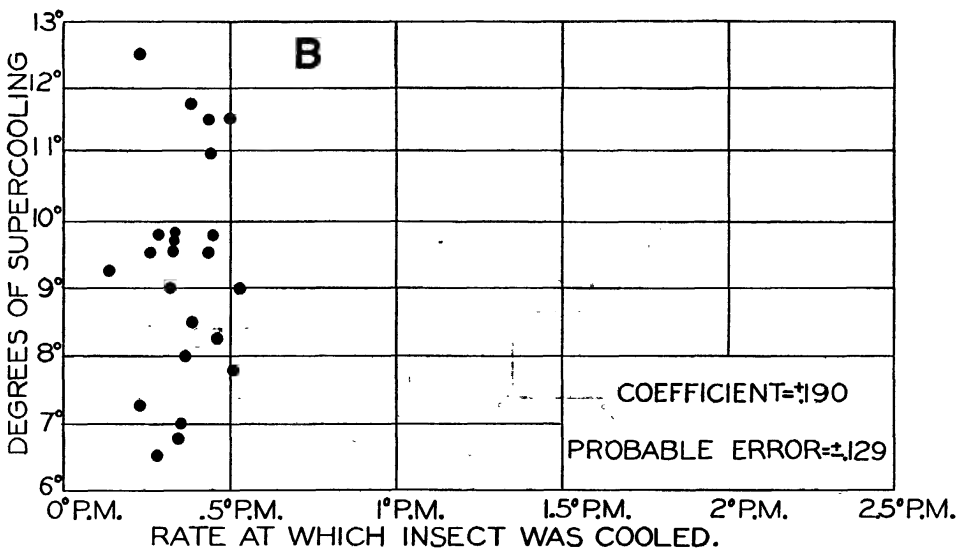
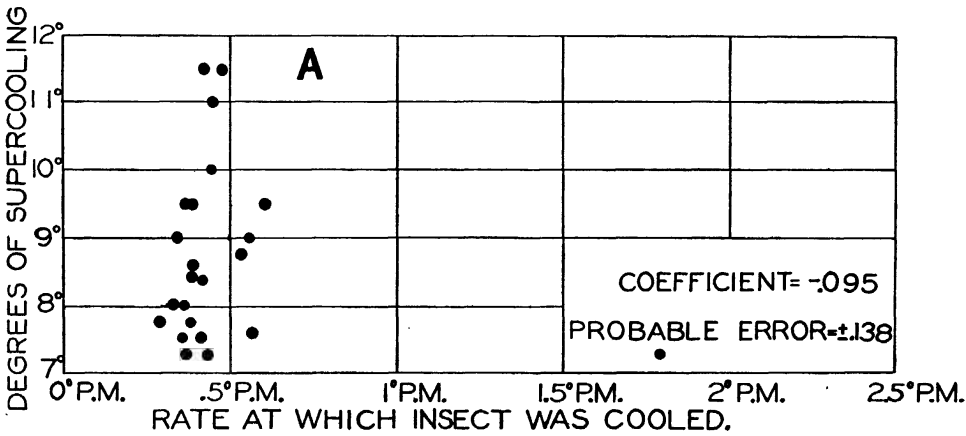


FIG. 3.—Relation between supercooling and the rate at which insect was cooled. A, adults; B, pupae; C, larvae.

Larson and Simmons (10) conducted an extensive series of experiments with commercial cold-storage conditions. They used three temperatures, 36, 32, and 20° F. Perfect control of the weevil infestation was obtained with 56 days' exposure to 32°. Loss of reproductive power was noted after exposure for 22 days or longer. Satisfactory control was obtained with 66 days' exposure at 36°. Unemerged adults were found to be the most resistant, pupae next, and larvae the least resistant. No experiments with eggs of *Bruchus obtectus* are recorded. It is suggested that weevils put into cold storage from lower temperatures obtaining in February could resist longer than those put in at the higher temperatures of November.

De Ong (3) used several species of insects in a study of cold-storage control. His findings are interesting here in that he reports that mature larvae were more resistant than any other stage studied. He also doubts the mortality of the egg at temperatures fatal to other more advanced stages.

METHOD

In these studies a constant-temperature cabinet was used with which it was possible to keep temperatures within less than 1° of the desired temperature for long periods. Glass vials containing beans infested with the several stages of weevil were placed in this cabinet and subjected to the temperatures as stated in the tables for the several periods.

A difficulty experienced in dealing with the immature stages of bean weevil infestation is that of knowing just which stage was being dealt with when the cavity first became visible from the outside of the bean. The cavity may contain either a pupa, prepupa, or even a larva. Another difficulty is in the fact that when beans are heavily infested with weevils in all stages the last larvae to enter the bean often perish through lack of food. This act rather tends to stress a larval mortality which may not be due to the conditions of the experiment. In order to avoid this colonies of known data were used for getting definite data on larvae. The most complete data, however, have been obtained from the use of beans where the weevils were showing black through the bean—in all cases unemerged adults—and those where the work of the weevil was visible from the outside, but the weevils were still cream-colored. The majority of these were early pupae, but larvae were sometimes found within these light-colored cells.

EFFECT OF FATAL TEMPERATURE ON THE APPEARANCE OF THE SEVERAL STAGES.

Eggs shrivel up and the contents become clear. It is not always easy to ascertain whether the eggs were killed by the temperature or were simply infertile. For this reason total mortality and hatching have been the criteria used. Pupae when fatally affected turn rotten and brown. The same is true of larvae. With unemerged adults later emergence was the criterion, and for active adults recovery.

An interesting condition met with was that called, for want of a better name, "arrested development." In this condition development proceeds for some time after return to normal temperatures, but the insect fails to mature and usually fails to complete the stage in which it was at the time of freezing. Pupae often continue their development until they are almost to the unemerged or quiescent

stage. The head and thorax and appendages can become complete, but the abdomen usually remains cream-colored, the insect dying at that point.

Unemerged adults show another phase of this condition. Normally the emerging beetles cut clean circles in the epidermis of the bean, but the treated unemerged adults lose the capacity to do this and endeavor to emerge by pushing through the center of the circle. They are found dead with part of the mouth parts showing through. In the upper limits of the zone of arrested development adults will continue development quite normally if assisted out of the bean. The effect here, then, seems to be an inhibiting of the normal response of the insect, resulting in death by imprisonment.

EXPERIMENTS ON TEMPERATURES FATAL TO *B. OBTECTUS*

Table III gives the results of experiments on the several stages, and following it are Figures 4, 5, and 6, in which the data of the experiment have been incorporated. The table has been condensed, but all the data are given in the figures.

TABLE III.—*Limits of fatal temperature-time zones of Bruchus obtectus*
ACTIVE ADULTS

Temperature	Time	Result	Temperature	Time	Result
° C.	Hours		° C.	Hours	
-2	168	Lived.		1	8 lived; 13 killed.
	24	Do.		2	Muscular activity, then death.
-7	36	Do.	-14	3	Do.
	48	1 lived.		4	Killed.
	24	Lived.		5	Do.
-8	30	Killed.	-20	2	Do.
	36	Do.		3	Do.
-9	1	Lived.			
	.5	Do.			
	1	Do.			
	2	18 lived; 10 killed.			
-10	3	Muscular activity, followed by death.			
	4-11	Do.			
	12	Killed.			
	13	Do.			
UNEMERGED ADULTS					
-8	24	Lived.	-14	1	Lived.
	36	Killed.		2	Killed.
	1	Lived.	-17	24	Do.
-10	2-6	Do.	-20	.25	Killed.
	7	Killed.		.50	Do.
	8	Do.			
PUPAE AND ADVANCED LARVAE					
-2	168	Lived.	-14	1	Lived.
	24	Do.		3-9	Killed. *
	36	Do.		24	Do.
-8	48	1 lived.	-17	36	Do.
	56	Killed. *		.5	Lived.
	60	Do.	-20	2	Killed. *
-9	1	Lived.		4	Do.
	1	Do.		6	Do.
	3-7	Do.			
	8	Killed. *			
-10	9-16	Do.			
	22-23	Do.			
	25-30	Do.			
	32-34	Do.			

* Cases where death followed post-freezing development.

TABLE III.—Limits of fatal temperature-time zones of *Bruchus obtectus*—Con.

LARVAE OF KNOWN AGE					
Temper- ature	Time	Result	Temper- ature	Time	Result
°C.	Hours		°C.	Hours	
—2	168	Lived.	—14	2	Killed.
—8	36	Killed.	—17	56	Do. *
—9	1	Do.	—20	24	Do.

EGGS					
—7	24 48	Hatched. Do.	—17	56 48	Killed. Do.
—8	56 60	Do. Killed.	—20	1 4	Hatched. Do.
—10	4 16	Hatched. Do.		5	Killed.
	2 5	Do. Killed.			
—14	6 6 7	Hatched. Killed. Do.			

* Cases where death followed post-freezing development.

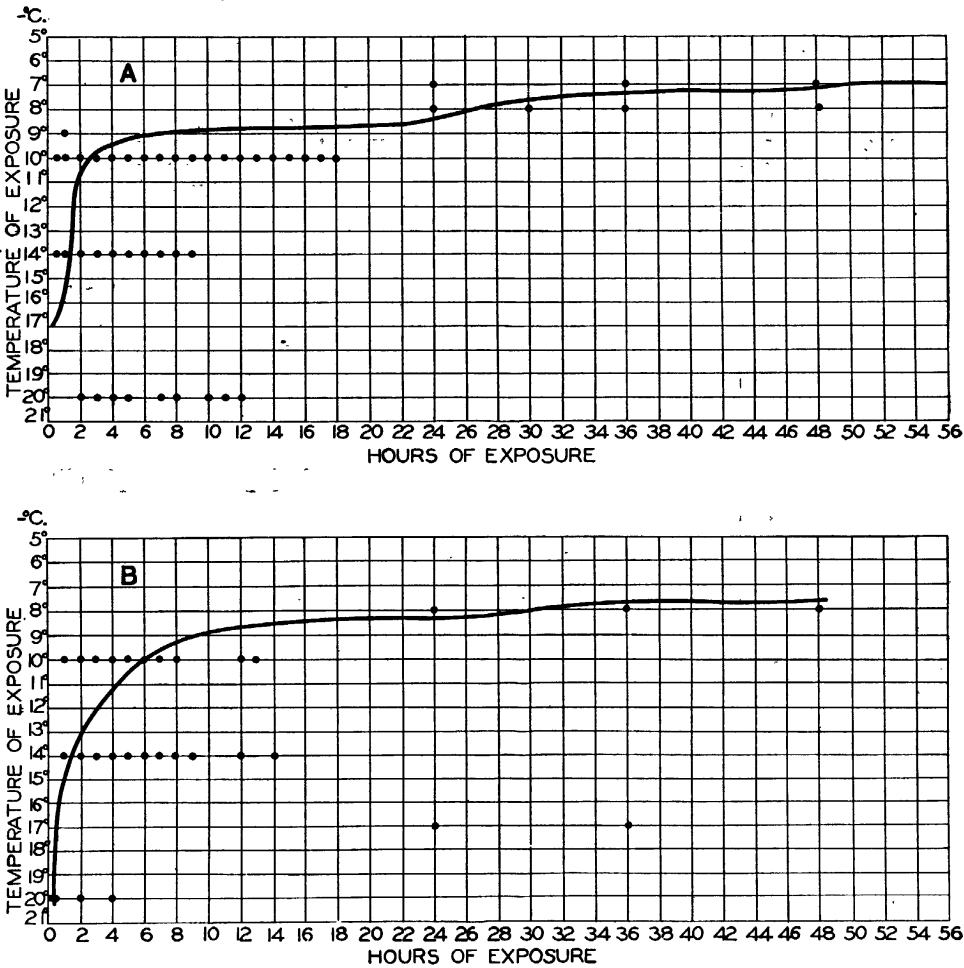


FIG. 4.—Limits of fatal temperature-time zone. A, active adults; B, unemerged adults

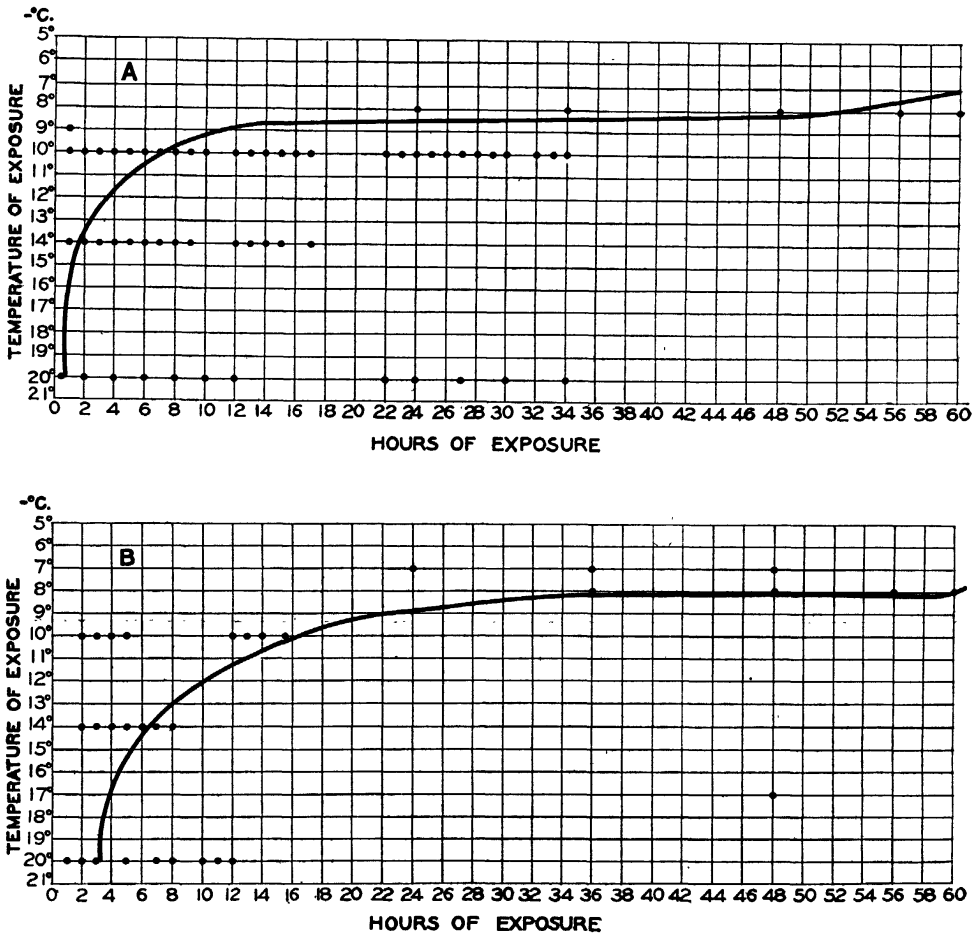


FIG. 5.—Limits of fatal temperature-time zone. A, pupae and advanced larvae; B, eggs

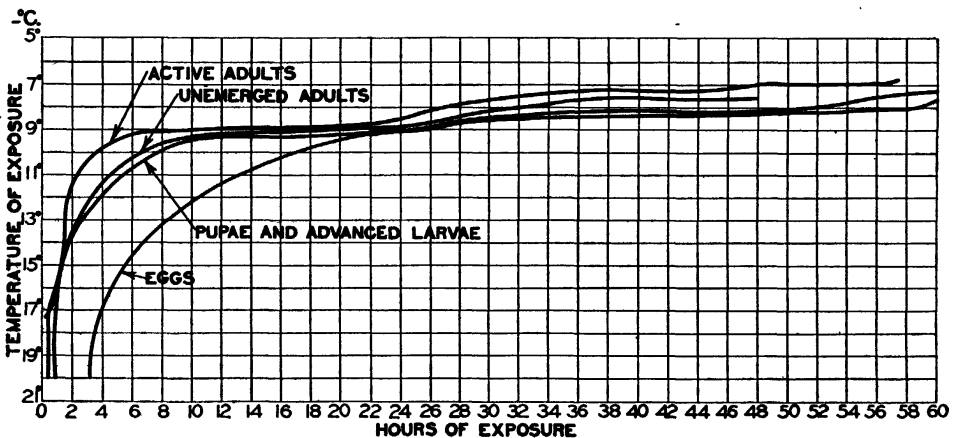


FIG. 6.—Fatal temperature-time zones for *Bruchus obtectus* Say. Active adults; unemerged adults; pupae and advanced larvae; eggs

From these data it is seen that active adults are slightly more susceptible to low temperatures. Unemerged adults and the stages designated as "pupae and advanced pupae" have curves practically similar. The slight difference is in favor of the latter. The eggs of *Bruchus obtectus* can apparently withstand lower temperatures than any other stage. With respect to eggs, however, the criterion used

was whether or not the eggs hatched, not the ultimate development of the insect.

These data further show that at temperatures down to -8°C . a much greater length of time must elapse before fatal results ensue. At that temperature all the curves flatten out at about the 24-hour period. In passing it should be noted that this temperature is approximately the one to which injured specimens supercool in the ether bath used in the electrothermal method.

The highest temperature used, -2°C . for one week, was not fatal to any stage, and Larson and Simmons (10) have shown that a much longer period than one week is necessary at 0°C . to insure a complete kill. The curve showing the limits of the fatal temperature-time zone is therefore very long and with a very gradual slope in the upper 8 degrees of below 0°C . temperature.

It is evident, however, that for lower temperatures a much shorter time is necessary. Referring to the curves again, it is seen that there was no survival at an exposure of -20°C . for a period of five hours, and even this period applies only to the hatching of eggs. Unemerged adults and pupae could withstand only a very short period at this temperature, probably only that period required to permit penetration of the temperature into the bean. Adults died at an exposure of -17° for less than one hour, while one hour at -14° was fatal to them.

From Table III it is seen that pupae and advanced larvae continue their development even after considerable periods of exposure. This is of much interest from the standpoint of physiology, but since such development does not continue long enough to permit the emergence of the insect, the curve has been drawn between the last points of survival and the first points indicating death without regard to whether there was any period of later development or not.

The data for larvae of known age are not as complete as those for the other stages. Table III indicates, however, that the mortality zone is the same as for unemerged adults and pupae and advanced larvae. As sets of vials containing larvae of several ages were used each time, it was possible to show that there was no difference in power of resistance between very young larvae and those that were well advanced, so that the mortality zone for pupae and advanced larvae can safely be applied to larvae of less development.

Larson and Simmons (10) suggest that the temperature at which the colony of weevils was maintained prior to freezing has some effect on resistance. To verify this a colony was maintained at approximately 0°C . for one week in a commercial cold-storage plant. At the end of this time adults, unemerged adults, and pupae were subjected to a temperature of 14° . The results indicate that no hardening had taken place. Both active and unemerged adults were killed by exposure for one hour, while exposures of two hours killed the pupae and advanced larvae. An exposure of seven hours killed pupae and advanced larvae at -10° . These results are essentially the same as with weevils taken from room temperatures.

SUMMARY

The common bean weevil, *Bruchus obtectus*, Say, has been used for experiments on supercooling and resistance to low temperature. Bachmetjew (2) has made the most extensive contributions to the subject of vital temperature in insects, and this study follows in a general way the lines laid down by him. While he believed that an insect's temperature must be lowered again to its supercooling point after a rebound has taken place before death ensued, subsequent works show that an insect is killed if a rebound occurs. A rebound is evidence that heat of crystalization is given off. Crystalization of the lymph is believed to be responsible for the death of the insect.

Comparing differences between individuals, it is found that pupae show more variation from the average trend. This is explained in the fact that pupae of all ages were used, and the physical properties of their lymph varied.

It has been seen that larval, pupal, and adult weevils could withstand lower temperatures when cooled uninjured than when the point of a thermocouple was thrust into them. Active adults withstood temperatures of -10° C., a temperature below the average supercooling point, for a period of three hours. Pupae and advanced larvae withstood the same temperatures for seven hours.

It has not been found possible to obtain the exact freezing point of *Bruchus obtectus* with present-day electrothermal methods.

There is a distinct correlation between the supercooling point and the rebound point, but this is believed to be due to radiation from the insect to the air of the cooling chamber. The exact freezing point is actually much higher, then, than the rebound points recorded.

The temperature-time experiments show that time is a factor in the resistance of *Bruchus obtectus* to low temperature. It is apparent, with freezing points as high as those of this insect must be, that supercooling takes place at temperatures of -10° C. or below. That being the case, the data imply that supercooling is possible for limited times only at certain temperatures.

The relationship between supercooling and the rate at which the insect has cooled has been studied. There seems to be no correlation between these two factors.

A condition of post-freezing development with ultimate death has been observed at temperatures just below the limits of resistance. This has been referred to in this paper as "arrested development." It manifests itself in the adults as failure to emerge, due to the loss of the insect's capacity to cut its way out of the bean; pupae can metamorphose only to adult form in the head and thoracic regions, the abdomen remaining undeveloped; larvae are unable to emerge from the egg, or, if they do emerge, it is often through the side of the egg, not through the operculum. Many that emerge from the egg are unable to enter the bean.

The limits of the fatal temperature-time zones have been determined for four stages of *Bruchus obtectus*. This insect apparently has no capacity for hardening.

The thermal constant, as described by Sanderson and Peairs (16), has not been worked out for this insect. A difference of less than 10° F.—the difference obtaining between the temperature of the laboratory and a cool, dry basement at about 64° —almost doubles the

length of time required for a complete life cycle. This means that even in the South development of the insect could be retarded considerably until fatal outdoor temperatures prevailed.

CONCLUSIONS

Bruchus obtectus Say dies if the heat of crystallization is given off. A definite correlation exists between supercooling and rebound, which is believed due to radiation. The electrothermal method does not give the true freezing point of an insect because of radiation and the effect of injury on the insect. There is no correlation between supercooling points and the time required to bring the insect to these points.

The capacity of *Bruchus obtectus* to resist low temperature is limited by the length of time it can remain in the supercooled condition. This capacity varies with the stage, active adults being least resistant and eggs most resistant.

Growth of *Bruchus obtectus* in beans can be materially retarded by storage in temperatures even as high as 64° F. Temperatures of below -10° C. for a period of 12 hours are fatal to all stages of this insect.

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THE INFLUENCE OF IRRIGATION WATER AND MANURE ON THE COMPOSITION OF THE CORN KERNEL¹

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INTRODUCTION

The total ash, calcium, magnesium, potassium, and phosphorus content of wheat, oats, and barley has been found to increase directly with the increase in quantity of irrigation water applied during the growing period (5).² This has been interpreted as being due to the increased bacterial activity resulting from an optimum moisture content (4) which would render available greater quantities of these specific elements to the growing plants. To test this theory, and also to obtain information concerning the composition of corn grown on highly calcareous soil, the work here reported was undertaken.

EXPERIMENTAL DATA

SOIL

The corn was grown on the Greenville Experiment Farm, the soil of which is of a sedimentary nature and contains large quantities of calcium and magnesium, probably in the form of dolomite. In chemical and physical composition it is very uniform to a great depth. The chemical and physical analyses of the soil are given in Table I. The chemical analyses were made according to the official methods of the Association of Official Agricultural Chemists (1), and the physical analyses were made by means of the Yoder (9) soil elutriator.

TABLE I.—*Chemical and physical composition of the soil of the Greenville experiment farm, North Logan, Utah*

Chemical composition		Physical composition	
Constituent	Per cent	Constituent	Per cent
Insoluble residue.....	41.46	Coarse sand.....	0.21
Soluble silica.....	.62	Medium sand.....	9.63
Total.....	42.08	Fine sand.....	30.04
Potash (K ₂ O).....	.67	Coarse silt.....	32.25
Soda (Na ₂ O).....	.35	Medium silt.....	12.30
Lime (CaO).....	16.88	Fine silt.....	6.25
Magnesia (MgO).....	6.10	Clay.....	7.63
Oxide of iron (Fe ₂ O ₃).....	3.03	Moisture.....	1.60
Alumina (Al ₂ O ₃).....	5.64	Soluble and lost.....	.10
Phosphoric acid (P ₂ O ₅).....	0.41	Specific gravity.....	2.67
Carbon dioxide (CO ₂).....	19.82	Apparent specific gravity.....	1.23
Volatile matter.....	5.6	Water-soluble salts.....	.06
Total.....	100.69		
Humus.....	.53		
Nitrogen.....	.14		

¹ Received for publication Oct. 14, 1924; issued September, 1925.

² Reference is made by number (*italic*) to "Literature cited," pp. 189.

The physical and chemical composition of this soil is quite uniform to a depth of 10 feet. The soil is exceptionally rich in phosphorus and potassium, but low in nitrogen and humus. The calcium and magnesium contents, mainly in the form of dolomite, are exceptionally high. The soil is especially fertile when supplied with optimum moisture and organic matter.

MANURE AND IRRIGATION

The plats on which the corn was grown were 7 feet wide and 24 feet long with a 4-foot walk dividing them. The land was plowed in the fall and left in that condition until spring when a mixture of fairly well-rotted horse and cow manure was applied to the various manured plats. In each ton of manure applied there were approximately 738 pounds of dry matter, 3.04 pounds of phosphorus, 13.7 pounds of potassium, and 16.08 pounds of nitrogen. The manure was thoroughly disked or plowed into the soil. Measured quantities of irrigation water were applied to the various plats as follows:

4 plats received no water and no manure.

2 plats received 5 inches of water and no manure; water applied in 2 equal applications.

2 plats received 10 inches of water and no manure; water applied in 2 equal applications.

2 plats received 20 inches of water and no manure; water applied in 4 equal applications.

2 plats received 30 inches of water and no manure; water applied in 6 equal applications.

2 plats received 40 inches of water and no manure; water applied in 8 equal applications.

On a duplicate series of plats, manure was applied at the rates of 5 and 15 tons to the acre, the application of water being the same in every way as that on the unmanured plats. Hence the corn was grown on soils (1) without manure, (2) with 5 tons of manure to the acre, and (3) with 15 tons to the acre. The water applied varied from none to 40 inches, both with and without manure. However, this does not represent the total water reaching the soil, for there was an annual average precipitation of about 18 inches which was the same for all plats.

The irrigation water contained in each million parts 144 parts of calcium carbonate, 78 parts of magnesium bicarbonate, 3 parts of magnesium chloride, 7 parts of magnesium sulphate, 8 parts of sodium bicarbonate, 2 parts of sodium sulphate, and 2 parts of sodium chloride. Each acre-foot of the water carried to the soil 2.2 pounds of potassium, 0.005 pound of phosphorus, and 1.1 pounds of sodium nitrate. Analyses of the soil showed that during the course of the experiment, little of the nitrogen (?) and phosphorus was being leached from the soil, but large quantities of the calcium magnesium and organic matter were disappearing. The analyses of the corn were made on composite samples taken from the various plats receiving the same treatment and from the 12 yearly yields from 1911 to 1922. The analyses were all made in duplicate according to the official methods (1).

NITROGEN

The percentage composition of the dry corn kernel and the pounds per acre of nitrogen in the grain receiving various manure and irriga-

tion treatments are given in Table II. In these results all grain grown without irrigation water and with the various manurial treatments is averaged together. Likewise, all the corn grown without manure and with varying irrigation treatments has been analyzed separately and then averaged in the table. Hence the results in all the tables represent the average analyses of the 12 years of corn and duplicate analyses from the grain grown on 12 different plats. Therefore the figures given should represent quite accurately the composition of the grain grown with these different treatments during the past 12 years.

TABLE II.—Percentages and pounds per acre of total nitrogen in the corn kernel grown with varying quantities of irrigation water and manure

Treatment	Nitrogen		Treatment	Nitrogen	
	Per cent	Pounds		Per cent	Pounds
No irrigation water ¹	2.07	78.71	40 inches irrigation water ¹	2.03	90.81
5 inches irrigation water ¹	2.04	91.34	No manure ²	1.94	70.29
10 inches irrigation water ¹	2.02	84.96	5 tons manure ²	2.07	95.09
20 inches irrigation water ¹	2.07	98.17	15 tons manure ²	2.13	102.63
30 inches irrigation water ¹	1.99	92.05			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

In these results there appears to be little difference in the nitrogen content of the corn kernel with varying quantities of irrigation water applied during the growing season. This is due to the manure, as an examination of the results from which the averages in the above table were obtained reveals the fact that the corn grown with no irrigation water contained 9 per cent more nitrogen than did that grown with 40 inches of water. The nitrogen content of the corn increases progressively with the manure applied. Therefore the nitrogen content of grain depends on the quantity of available nitrogen in the soil. Although up to a certain point the speed with which nitrogen is made available is dependent upon the moisture content of the soil (4), the quantity which remains within the feeding area of the plant rather rapidly decreases as the water applied increases, because of its great solubility (3). Therefore the high protein content of dry-farm grains is due to two factors: (1) The storage of two years' nitrogen in the surface soil due to summer-fallowing (3); (2) the scant rainfall permitting the nitrates formed to remain and through upward capillarity to be concentrated in the surface-foot sections. Hence the nutrient media on which the plant is feeding is high in nitrates and the plant consequently builds protoplasm having a high nitrogen concentration.

Slightly larger quantities of total nitrogen are removed in the corn kernel from the irrigated soil than from the nonirrigated. However, there is no uniformity in the results in this respect. The grain grown on the manured soil, on the other hand, shows a relationship between the nitrogen applied and that removed in the crop. Thirty-one per cent of the added nitrogen was accounted for in the corn kernel grown on the soil receiving 5 tons of manure yearly, and only 14 per cent where 15 tons to the acre were applied; but the excess applied in the 15-ton application accumulates (6) in the soil, and if the soil is not manured for a number of years it yields much of this remaining nitrogen to the grain grown later.

ASH

The average percentage composition and pounds to the acre of total ash in the corn kernel grown with varying quantities of irrigation water and manure are given in Table III.

The corn kernel grown with 40 inches of irrigation water contains 8 per cent more ash than the corn kernel grown with no irrigation water, but the ash is as high with 10 inches of water as with 40. The ash content is highest where the soil receives enough moisture to promote maximum bacterial activity with the resulting liberation of the maximum of plant food.

TABLE III.—Percentages and pounds per acre of total ash in the corn kernel grown with varying quantities of irrigation water and manure

Treatment	Ash	Nitrogen per acre	Treatment	Ash	Nitrogen per acre
	<i>Per cent</i>	<i>Pounds</i>		<i>Per cent</i>	<i>Pounds</i>
No irrigation water ¹	1.65	62.46	40 inches irrigation water ¹	1.79	76.79
5 inches irrigation water ¹	1.66	74.05	No manure ²	1.65	58.96
10 inches irrigation water ¹	1.79	75.39	5 tons manure ²	1.75	82.20
20 inches irrigation water ¹	1.78	81.73	15 tons manure ²	1.76	84.74
30 inches irrigation water ¹	1.76	81.39			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

That it is the available plant food which determines the ash content of the grain is well brought out by the results with the manurial treatments. The grain grown on soil receiving 5 tons of manure per acre yearly contains 6 per cent more ash than corn grown under similar conditions but receiving no manure. That grown on soil receiving 15 tons of manure yearly contains only slightly more ash than that grown with 5 tons of manure.

The quality of ash in the corn kernel removed from the soil increases as the water applied increases up to 20 inches; above this there is a decrease. That removed by the plant markedly increases with increase in manure.

PHOSPHORUS

The phosphorus content of the corn kernel increased progressively with the irrigation water applied (Table IV). The kernel of copiously irrigated corn contained more than 9 per cent more phosphorus than did that of the unirrigated. The percentage might have been still higher if greater quantities of water had been applied (5). Unirrigated corn has been found to be higher in phosphorus than unirrigated wheat, oats, or barley. However, irrigated wheat, oats, and barley were higher in phosphorus than the highly irrigated corn. The increase due to the use of manure is very small.

With one exception the quantity of phosphorus removed from the soil by the corn grain increased as the water applied increased up to 30 inches. The quantity removed from the manured plats increased as the manure applied increased. The increase due to manure was greater than the increase due to water. The lowest phosphorus content of the corn kernel, as grown on this soil, is 29 per cent higher than the average reported by Hopkins (8, *p.* 603) after von Wolff; the highest is 42 per cent higher than the average of von Wolff. These results and those previously published (5) make it certain that

grains grown on this soil are exceptionally rich in phosphorus and that the phosphorus content of grain is increased by irrigation water, the increase being due primarily to a greater supply of available phosphorus.

TABLE IV.—Percentages and pounds per acre of total phosphorus in the corn kernel grown with varying quantities of irrigation water and manure

Treatment	Phos-phorus	Phos-phorus per acre	Treatment	Phos-phorus	Phos-phorus per acre
	<i>Per cent</i>	<i>Pounds</i>		<i>Per cent</i>	<i>Pounds</i>
No irrigation water ¹	0.32	12.12	40 inches irrigation water ¹35	15.54
5 inches irrigation water ¹33	14.50	No manure ²33	11.84
10 inches irrigation water ¹33	13.69	5 tons manure ²34	16.02
20 inches irrigation water ¹33	15.95	15 tons manure ²33	16.11
30 inches irrigation water ¹35	16.13			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

CALCIUM

The calcium content of the same grain is given in Table V.

TABLE V.—Percentages and pounds per acre of calcium in the corn kernel grown with varying quantities of irrigation water and manure

Treatment	Calcium	Calcium per acre	Treatment	Calcium	Calcium per acre
	<i>Per cent</i>	<i>Pounds</i>		<i>Per cent</i>	<i>Pounds</i>
No irrigation water ¹	0.13	4.77	40 inches irrigation water ¹	0.14	6.09
5 inches irrigation water ¹18	8.08	No manure ²14	4.81
10 inches irrigation water ¹18	7.01	5 tons manure ²15	6.12
20 inches irrigation water ¹14	6.89	15 tons manure ²15	8.15
30 inches irrigation water ¹14	5.31			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

The percentage of calcium in the corn kernel increases with the irrigation water applied up to 10 inches yearly; above this it gradually decreases. Corn grown with 10 inches of irrigation water contains 40 per cent more calcium than corn grown without irrigation water; that receiving 40 inches of irrigation water contains only 8 per cent more calcium. Manure also increases the calcium content of the corn, but not to the same extent as does irrigation.

The calcium-low corn in this series contains about six times the quantity reported by Hopkins (8, p. 603), whereas the calcium-rich corn contains nearly nine times the amount. Differences such as this must be of great significance in animal nutrition. Moreover, the calcium-phosphorus ratio changes with the application of irrigation water. In the corn grown with irrigation water the calcium-phosphorus ratio is 1:2.5, that grown with 10 inches of irrigation water is 1:1.8, whereas that grown with 40 inches has a ratio of 1:2.6. The application of manure also narrows the calcium-phosphorus ratio. It appears from these results, viewed from the standpoint of the mineral elements, that corn grown on this soil with 10 inches of irrigation water is the most valuable from the viewpoint of the nutrition of animals.

MAGNESIUM

The average magnesium content of the same corn is given in Table VI.

TABLE VI.—Percentages and pounds per acre of total magnesium in the corn kernel grown with varying quantities of irrigation water and manure

Treatment	Magnesium	Magnesium per acre	Treatment	Magnesium	Magnesium per acre
	<i>Per cent</i>	<i>Pounds</i>		<i>Per cent</i>	<i>Pounds</i>
No irrigation water ¹	0.20	7.57	40 inches irrigation water ¹	0.20	8.92
5 inches irrigation water ¹20	9.59	No manure ²19	6.72
10 inches irrigation water ¹20	7.98	5 tons manure ²22	10.11
20 inches irrigation water ¹20	9.65	15 tons manure ²21	9.58
30 inches irrigation water ¹21	9.12			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

The magnesium content of the corn is nearly constant with all water treatments. The magnesium content of this corn is nearly twice that reported by von Wolff (8, p. 603). Even with the high magnesium content of this grain grown on unmanured soil, manured grain shows a gain in magnesium content of 15 per cent.

The calcium-magnesium ratio of corn is 1:1.37. The total quantity of calcium and magnesium removed from the soil increases with the irrigation water and manure applied. The results all tend to bear out the theory that the change in the composition of the grain is due primarily to an increased available supply of plant food resulting from increased bacterial activity. This in turn is a function of the moisture and organic content of the soil (2, 4).

POTASSIUM

The potassium content of the corn kernel is given in Table VII.

TABLE VII.—Percentages and pounds per acre of total potassium in the corn kernel grown with varying quantities of irrigation water and manure treatment

Treatment	Potassium	Potassium per acre	Treatment	Potassium	Potassium per acre
	<i>Per cent</i>	<i>Pounds</i>		<i>Per cent</i>	<i>Pounds</i>
No irrigation water ¹	0.37	14.62	40 inches irrigation water ¹	0.41	18.09
5 inches irrigation water ¹39	16.42	No manure ²38	13.46
10 inches irrigation water ¹40	16.60	5 tons manure ²39	18.27
20 inches irrigation water ¹40	19.38	15 tons manure ²41	19.72
30 inches irrigation water ¹40	17.78			

¹ And varying quantities of manure.

² And varying quantities of irrigation water.

The potassium content of the corn kernel increases with increase of the water applied during the growing season. Corn grown with 40 inches of irrigation water contains 12 per cent more potassium than that grown without irrigation water; that grown with 15 tons of manure contains 8 per cent more potassium than that grown without manure.

SUMMARY

Corn grown on a highly calcareous soil with and without irrigation water and manure showed a decrease in the nitrogen content of the kernel due to the irrigation water and an increase due to the manure, the nitrogen content of the grain being a function of the available nitrogen content of the soil. Although irrigation water increases the speed with which nitrogen is rendered soluble in the soil, the water carries the readily soluble nitrogen beyond the feeding area of the plant. Hence the nitrogen available to the plant, proportionately to the requirements, is greater under nonirrigation than under irrigation.

The ash, calcium, phosphorus, and potassium contents are increased with the application of irrigation water. All the constituents are greatly increased by manure. The results all bear out the idea that the increased ash and mineral constituents of the grain are due to an increased bacterial activity which increases the available plant food in the soil.

The increased calcium, potassium, and phosphorus and the narrower calcium-phosphorus ratio in the corn kernel grown with irrigation water make such corn more valuable for human and animal nutrition than similar corn grown without irrigation water.

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CLEANING MILKING MACHINES¹

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INTRODUCTION

This paper gives the results of tests made for the purpose of comparing heat, chlorine, and salt and chlorine as agents for sterilizing milking machines. The word sterilizing is here used not in its technical meaning, but in the popular sense of denoting treatment which supposedly kills the organisms present with such a degree of completeness as to render the apparatus sanitary. In view of the various methods advocated for the sterilization of milking machines,² some comparative studies on three of the methods most generally used were considered desirable.

METHODS OF STERILIZING

Three single units were used in these tests. The units were washed and sterilized by the writer personally during the entire period, each unit receiving exactly the same treatment except in the sterilizing.

Immediately after milking, each unit was rinsed by drawing clean cold water through by vacuum, then it was washed with a brush in hot water (110° to 120° F.) containing washing powder, and then rinsed in clean hot water. Each unit (consisting of teat cups, claw, and milk tubes) was taken apart and thoroughly washed after each fourth or fifth milking. The units were sterilized as follows:

One unit was placed in a 5-gallon crock containing a chloride of lime solution (1 part available chlorine to 5,000 parts water), and one in a 5-gallon crock containing a saturated brine solution and chloride of lime (1 part available chlorine to 5,000 parts brine). These two units remained in the solutions for 24 hours or longer between milkings, except for the short period when they were used twice a day. Fresh solutions were made up after using for 8 or 10 milkings in cold weather, and after 4 or 5 in warm weather. The third unit was placed in hot water at 160° to 165° F. for 20 to 30 minutes just before milking, being kept in clean cold water the rest of the time.

A stock solution was made by dissolving a 12-ounce can of chloride of lime in 1 gallon of water and filtering into a glass bottle or jar. This was covered and kept in a cool, dark place. Sterilizing solutions were made by adding 1 ounce of stock solution to every gallon of water used.

¹ Received for publication October 21, 1924; issued September, 1925.

² RUEHLE, G. L. A., BREED, R. S., and SMITH, G. A. MILKING MACHINES. N. Y. State Agr. Exp. Sta. Bul. 450: 113-181, illus. 1918.

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Each unit was given an identification mark through the tests. The milk buckets were sterilized with steam after washing. The units were used but once a day during the greater period of the tests, which ran for six months. Three or four cows were milked with each unit at a milking. As far as possible, the same cows were milked during the entire period. Also, the machines were rotated—i. e., a machine which milked one group of cows one day was used to milk another group the next day and so on—so that each machine milked the same cows about an equal number of times. Cow's teats and udders were washed and wiped with a clean cloth before milking. All cows chosen were normally low in udder bacteria.

Results of the tests are shown in Tables I, II, and III.

TABLE I.—Summary of bacterial counts obtained, in cold weather, on samples of milk drawn with machines sterilized by three different methods

Method	Number of sam- ples of milk	Range in bacterial count per c. c.	Average bacterial count per c. c.	Samples of milk having a bacterial count of 10,000 per c. c. or lower	
				Number	Per cent of total
Heat.....	239	200 to 12, 000	2, 390	238	99. 6
Chloride of lime.....	241	900 to 25, 000	5, 130	222	92. 1
Salt and chloride of lime.....	241	800 to 24, 000	5, 250	218	90. 5

TABLE II.—Summary of bacterial counts obtained, in warm weather, on samples of milk drawn with machines sterilized by three different methods

Method	Number of sam- ples of milk	Range in bacterial count per c. c.	Average bacterial count per c. c.	Samples of milk having a bacterial count of 10,000 per c. c. or lower	
				Number	Per cent of total
Heat.....	103	600 to 6, 700	2, 330	103	100. 0
Chloride of lime.....	103	2,700 to 160, 000	17, 300	59	57. 3
Salt and chloride of lime.....	104	2,100 to 45, 000	8, 460	78	75. 0

TABLE III.—Summary of all bacterial counts (Tables I and II combined)

Method	Number of sam- ples of milk	Range in bacterial count per c. c.	Average bacterial count per c. c.	Samples of milk having a bacterial count of 10,000 per c. c. or lower	
				Number	Per cent of total
Heat.....	342	200 to 12, 000	2, 380	341	99. 7
Chloride of lime.....	344	900 to 160, 000	8, 780	281	81. 7
Salt and chloride of lime.....	345	800 to 45, 000	6, 220	296	85. 8

As noted in Tables I, II, and III, the average bacterial counts on samples of milk drawn with the machine which was sterilized by the heat method were all very uniform, only one count going higher than 10,000 per c. c. There was very little difference between the results obtained by the chloride of lime method and by the brine or salt and chloride of lime method, during the cold weather. The results were very good, although not so good as those obtained by the heat method. During the warm weather, however, the bacterial counts accompanying the chloride of lime method and the salt and chloride of lime method rose considerably. Of the two methods the salt and chloride of lime method gave much better results than the chloride of lime. This was evidently due to the presence of the salt, which undoubtedly acted as a stabilizer in keeping the available chlorine from being given off too rapidly. However, the salt and chloride of lime had a marked corrosive action on some of the metal parts of the milker, which the heat method and plain chloride of lime solution did not have.

For a short period during one month the machines were used for two milkings a day, and during this time the unit which was sterilized with heat was placed in the hot water (160° to 165° F.) after it was washed and allowed to remain there until the next milking, the water cooling off gradually. The other two units were treated as formerly, being placed in their respective solutions after they were washed, and allowed to remain there until the next milking. Seventy-five samples of machine-drawn milk from each unit were collected during the month, 45 of which were from once-a-day milking and 30 from twice-a-day milking. Results are given in Table IV.

TABLE IV.—Comparison of bacterial counts from milking once and twice a day

Method	Once-a-day milking		Twice-a-day milking	
	Number of samples of milk	Average bacterial count per c. c.	Number of samples of milk	Average bacterial count per c. c.
Heat.....	45	2,380	30	2,450
Chloride of lime.....	45	5,220	30	5,160
Salt and chloride of lime.....	45	5,280	30	5,240

As noted in Table IV, there was practically no difference between the bacterial counts of the once-a-day and twice-a-day milking samples under the same sterilizing treatment.

All units were used for about 120 milkings, no rubber parts were replaced on any of them during this time, and all were still in good condition at the end of the experiment. Bacterial counts were made on samples of the sterilizing solutions of chloride of lime, and salt and chloride of lime, taken from the long milk tube. These counts varied considerably. There was no correlation between the age or bacterial counts of the solutions so taken and of the bacterial counts of the milk samples taken on the same days during the cold weather. During the warm weather, however, there was a direct relation (with one exception) between the age of the solution and the bacterial counts of the milk: the older the solution the higher the counts of the milk. A summary of the results is shown in Tables V and VI.

TABLE V.—Comparison of age and bacterial count of sterilizing solution taken from long milk tubes, with bacterial count of the milk. (During cold weather)

Chloride of lime				Milk		
Age of solution, in the number of the milking for which used	Number of samples	Range in bacterial count	Average bacterial count per c. c.	Number of samples	Range in bacterial count	Average bacterial count per c. c.
First.....	2	0	0	6	1,700 to 25,000	9,430
Second.....	4	0 to 3	2	12	1,600 to 10,200	3,730
Third.....	3	0 to 4	2	9	1,100 to 12,000	4,840
Fourth.....	4	0 to 4	1	12	900 to 11,000	4,670
Fifth.....	5	0 to 1	1	15	2,700 to 13,300	5,360
Sixth.....	2	0 to 1	1	6	2,100 to 9,800	5,070
Seventh.....	3	0 to 2	1	9	2,200 to 14,000	4,330
Eighth.....	7	0 to 300	44	21	1,400 to 11,400	4,710
Ninth.....	4	0 to 10	3	12	1,100 to 8,400	4,970
Tenth.....	3	1 to 20	7	9	2,500 to 11,000	5,410

Salt and chloride of lime solution				Milk		
Age of solution, in the number of the milking for which used	Number of samples	Range in bacterial count	Average bacterial count per c. c.	Number of samples	Range in bacterial count	Average bacterial count per c. c.
First.....	3	0 to 3	1	8	1,200 to 3,900	2,860
Second.....	3	0	0	9	800 to 6,800	3,500
Third.....	3	0 to 7	3	11	1,200 to 12,500	4,940
Fourth.....	4	1 to 4	3	12	1,000 to 11,000	4,280
Fifth.....	6	0 to 400	69	18	1,600 to 12,000	4,950
Sixth.....	3	0 to 3	1	9	1,100 to 9,100	5,870
Seventh.....	4	0 to 8	3	12	1,600 to 20,000	5,340
Eighth.....	6	0 to 250	63	18	1,400 to 12,600	5,880
Ninth.....	4	0 to 440	115	12	1,800 to 12,800	5,480
Tenth.....	2	0 to 40	20	6	1,500 to 8,800	4,500

TABLE VI.—Comparison of age and bacterial count of sterilizing solution taken from long milk tubes, with bacterial count of the milk. (During warm weather)

Chloride of lime solution				Milk		
Age of solution, in the number of the milking for which used	Number of samples	Range in bacterial count	Average bacterial count per c. c.	Number of samples	Range in bacterial count	Average bacterial count per c. c.
First.....	5	0 to 200	44	15	2,700 to 19,300	8,410
Second.....	7	1 to 20	5	21	4,200 to 42,000	11,840
Third.....	7	1 to 5,000	859	21	3,800 to 63,000	14,540
Fourth.....	7	2 to 48,000	11,004	21	3,400 to 160,000	23,970
Fifth.....	5	20 to 10,000	2,286	15	5,300 to 128,000	24,870

Salt and chloride of lime solution				Milk		
Age of solution, in the number of the milking for which used	Number of samples	Range in bacterial count	Average bacterial count per c. c.	Number of samples	Range in bacterial count	Average bacterial count per c. c.
First.....	6	0 to 12	4	18	2,500 to 9,400	4,630
Second.....	7	1 to 30	10	21	2,100 to 24,400	7,970
Third.....	7	0 to 180	37	21	3,100 to 36,000	9,350
Fourth.....	7	1 to 1,440	433	21	3,500 to 45,000	11,030
Fifth.....	5	10 to 720	215	15	3,100 to 28,000	10,460

SUMMARY AND CONCLUSIONS

Comparative tests of the three methods commonly used for sterilizing milking machines—namely, heat, chloride of lime, and a mixture of common salt and chloride of lime—showed that the heat method, under conditions as nearly identical as possible, gave more uniform and appreciably lower bacterial counts than did the other two methods.

There was practically no difference in the results obtained by the chloride of lime method and the salt and chloride of lime during the colder weather. About 92 per cent of the samples of milk drawn with the unit sterilized in the chloride of lime solution had bacterial counts of 10,000 per c. c. or lower. About 90 per cent of the samples of milk drawn with the unit sterilized in the salt and chloride of lime solution had bacterial counts of 10,000 per c. c. or lower.

Neither of these two methods gave as good results in warm weather as they did in cold weather; and the salt and chloride of lime method gave much better results than did the plain chloride of lime. Only about 57 per cent of the samples of milk drawn with the unit sterilized in the chloride of lime solution had a bacterial count of 10,000 per c. c. or lower, and about 75 per cent of the samples drawn with the unit sterilized with the salt and chloride of lime solution had a count of 10,000 per c. c. or lower. The salt and chloride of lime solution corroded some of the metal parts.

The results obtained in these experiments were chiefly concerned with once-a-day milking; check samples taken from twice-a-day milking, however, showed practically no difference, there being a variation of less than 100 bacteria per c. c. in the averages of once-a-day and twice-a-day milking for each method. During this period when samples were taken twice a day the unit sterilized with heat was allowed to remain in the hot water until the next milking, the water cooling gradually. The other two units were treated as formerly, being placed in their respective sterilizing solutions after they were washed, and allowed to remain there until the next milking.

Bacterial counts made on samples of the sterilizing solutions taken from the long milk tubes of the two units sterilized in the chloride of lime and the salt and chloride of lime solutions, varied considerably.

In cold weather, there was no correlation between the age or bacterial counts of the solutions and the bacterial counts of the milk samples taken on the same days. During the warm weather, however, there was a direct relation (with one exception) between the age of the solution and the bacterial count of the milk; the older the solution, the higher the bacterial count of the milk.

The experiments indicated that making up a fresh sterilizing solution of chloride of lime, or of salt and chloride of lime, once a week in cold weather, would undoubtedly be sufficient to insure a good grade of milk, if the units were thoroughly washed and the solution kept free from all foreign matter. During warm weather, however, fresh chloride of lime solution should be made daily, even under the most cleanly conditions; and new salt and chloride of lime solutions should be made up about every other day.

LABORATORY TESTS ON EFFECT OF HEAT ON SEEDS OF NOBLE AND SILVER FIR, WESTERN WHITE PINE, AND DOUGLAS FIR ¹

By J. V. HOFMANN

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To test the ability of the seed of noble fir (*Abies nobilis*) and silver fir (*A. amabilis*) to withstand the effects of forest fires, samples of seed were subjected for 10 hours each to dry heat of varying temperatures from 100° to 300° F. and to moist heat of 100° to 240°. The samples consisted of 225 seeds for each test. The moist condition was produced by keeping water in the heating oven to maintain the air as near the point of saturation as possible.²

Duplicate tests with Douglas fir (*Pseudotsuga taxifolia*) and western white pine (*Pinus monticola*) were made at the same time.

After heating, 25 seeds were taken from each sample for microscopic examination, and the remaining 200 seeds were sown in the nursery in drills, a separate sample in each drill.

The seeds for these germination tests were sown in the nursery at the end of August. A little germination appeared in the fall of that year, but most of it occurred during the following season. The results are shown in Table I.

TABLE I.—Total germination from samples of 200 seeds each of noble, silver, and Douglas fir, and western white pine heated for 10 hours

DRY HEAT

Temperature (° F.)	Noble fir	Silver fir	Douglas fir	Western white pine
	Number	Number	Number	Number
100.....	32	3	128	174
140.....	0	1	124	191
160.....	9	0	123	157
180.....	3	0	146	167
200.....	0	0	1	47
220.....	1	0	0	0
240.....	0	0	0	0
Not heated.....	4	3	110	188

MOIST HEAT (AIR SATURATED)

100.....	34	0	130	167
140.....	11	16	120	165
160.....	0	0	48	97
180.....	0	0	0	0
200.....	0	0	0	0
220.....	0	0	0	0
240.....	0	0	0	0
Not heated.....	4	3	110	188

¹ Received for publication Oct. 28, 1924; issued September, 1925.

² For heating in moist air above 240° a pressure oven is required.

The inferior quality of the true fir seed renders these tests mainly of interest in showing results for the pine and Douglas fir. For these species the limit of heat under which the seeds remained viable was between 200 and 220° F. dry heat and considerably below that for moist heat. The fact, however, that one true fir seed germinated after the application of 220° of dry heat shows at least the possibility of seeds retaining their vitality under this temperature for several hours.

From the fact that only one seed withstood intense heat successfully one might conclude that seed subjected to the heat of forest fires in the cone (which would in some degree be moist heat) has small likelihood of retaining its viability. It must be remembered, however, that cones scorched by an ordinary crown flash are heated for only a short time, so that the seed rarely reaches very high temperatures.

On the other hand, the comparatively low temperatures of the 10-hour tests that resulted in appreciable germination indicate that in a crown fire of devastating intensity there is small chance for the seed of any of these species to survive solely through the protection of cones while on the trees, although it may be possible that some seed will escape the fire in this way.

Observations of the condition of the seeds during the heating tests may be briefly detailed as follows:

SILVER FIR

The silver fir seed in its normal condition has a glistening endosperm; brownish, translucent seed coats; and a smooth surface. The small blisters in the exocarp are well filled with pitch. When the seed was subjected to dry heat there was no apparent change until 140° F. was reached, when a slight drying of the seed was noticeable. At 160° the pitch began to ooze from the seed coats and the endosperm was slightly whiter. As the temperature rose higher, this drying of the endosperm became more and more apparent. At 200° so much pitch had exuded from the seeds that they began to stick together. This condition progressed until, at 300° of dry heat, the seeds browned, and so much pitch had oozed out that they were stuck to the dish.

With the seed given moist heat, the only apparent change was the oozing of the pitch until, at the highest temperature, 240° F., the seeds stuck to one another, but not so much so as in the dry-heated seeds. No change in the endosperm in drying or in color was apparent.

NOBLE FIR

The normal noble fir seed has a smooth exocarp, with small pitch pockets well filled with pitch. The seed coats are translucent when taken separately; and the endosperm has a glistening color, but it usually does not fill the ovary. When the seed of the noble fir was dry heated, there was no change apparent up to 160° F. Then the exocarp turned darker and shiny, and this condition became more marked with the continued rise of temperature. At 180° there was an appreciable drying of oils in the endosperm. These conditions became more noticeable until, at 300°, the seed was shriveled, and a great deal of pitch exuded and the endosperm was darkened.

In the moist-heat series this change of color was not so apparent, and the shriveling of the seed did not occur.

WESTERN WHITE PINE

The normal western white pine seed has an exocarp with a slight grayish bloom; the endosperm is very oily and smooth and fills the entire ovary. No changes were apparent until 160° F. was reached, when the exocarp began to darken, and the pitch began to ooze from the seed. These changes became more and more apparent as the temperatures became higher. At 200° drying of the endosperm became noticeable. At 240° so much pitch had oozed from the seed coats that the seeds stuck together and stuck to the dish. At 300° the exocarp was very brown and almost charred where in contact with the dish, while the drying of the endosperm was marked.

The seeds subjected to moist heat did not show these changes of color or shriveling of endosperm, but the amount of oozed pitch was about the same.

DOUGLAS FIR

The normal Douglas fir seed has a mottled, dark-brown exocarp and a whitish clear endosperm which entirely fills the ovary. There was no change apparent until 180° F. was reached, when the exocarp became slightly darker, and shiny. This became more noticeable until, at 240°, the seeds had exuded enough pitch to stick together and stick to the dish. The endosperm did not show any change until 240°, when it was noticeably drying out and slightly shriveled. At 300° the exocarp was a very dark brown, the endosperm was noticeably shriveled, and a great deal of pitch had oozed out.

The seeds in the moist-heat series did not show this shriveling of the endosperm, and the change of color in the exocarp was not so marked, although about the same amount of pitch came from the seeds.



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No. 3

VITAMIN A IN BEEF, PORK, AND LAMB¹By RALPH HOAGLAND, *Senior Biochemist*, and GEORGE G. SNIDER, *Chemical Laboratorian, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Until comparatively recently the term "vitamin A" has been used synonymously with "fat-soluble A" to denote that vitamin which is found most abundantly in cod-liver oil, egg yolk, butter fat, liver, and certain other products. It is now believed, however (2, 5, 6, 7),² that fat-soluble A really consists of two vitamins. One of these is necessary for growth in young animals, its absence being indicated by nutritive failure, by the development of ophthalmia, and by certain other symptoms. The other is essential for the normal development of bones, and its absence is denoted by a disturbed calcium and phosphorus metabolism resulting in the onset of rickets. The first of these fat-soluble vitamins is now designated as vitamin A, while the other is commonly known as the antirachitic vitamin. It is with the first of these vitamins, vitamin A, that this paper is concerned.

Adequate information concerning the distribution of vitamin A among our foodstuffs is much to be desired in order that such foods as will provide an abundance of this vitamin may be selected for the diet. Since meat is one of our most important food products, it is desirable to have more information regarding its value as a source of vitamin A than is now available. It is important, also, to know the extent to which vitamin A is stored in the muscles of the ox, the sheep, and the hog, because of the bearing that such reserves have upon the vitamin A requirements of those animals.

Although meats are generally considered to be very deficient in vitamin A, it appears that relatively little systematic work has been done on the subject. McCollum, Simmonds, and Parsons (4) found that rats made very poor growth when fed rations containing even very large proportions of dried lean beef as the source of vitamin A. They conclude that "muscle tissue is very deficient in these vitamins but does not entirely lack any one of them."

Wright (8) determined the vitamin A content of pork that had been held in cold storage for nine years, the feeding tests being carried on with young chickens. The amount of pork fed to each group of chickens is not stated, but the birds that were fed the rations containing pork as the source of vitamin A made practically as good growth as those fed a normal ration.

The purpose of this investigation was to determine the vitamin A content of beef, pork, and lamb as measured by the growth induced in young albino rats when fed a ration adequate in other respects but containing dried lean meat as the sole source of vitamin A.

¹ Received for publication August 28, 1924; issued September, 1925. The authors extend their thanks to Oliver P. Clipper, assistant laboratorian, for assistance rendered in conducting the feeding tests.

² Reference is made by number (*italic*) to "Literature cited," p. 221.

EXPERIMENTAL WORK

DESCRIPTION AND CARE OF BREEDING RATS

The albino rat was used in all the tests reported in this paper. The foundation stock for the breeding colony was obtained from E. C. Schroeder, in charge of the Experiment Station of the Bureau of Animal Industry, United States Department of Agriculture, at Bethesda, Md. A continual process of selection and elimination has been practiced in breeding the rats, and the colony is healthy, vigorous, and prolific. The average weight of mature males is approximately 300 grams and of females 190 grams. The number of young in a litter ranges from 4 to 16, the average being approximately 8. The rats, both breeding and experimental, are kept in a well-lighted room with a southern exposure; but, on account of certain obstructions in front of the windows, only a very few of the rats are exposed to the direct rays of the sun.

The following-described ration is at present being fed to the breeding rats, and it has not been modified materially for some time:

Ration for breeding rats

	Per cent		Per cent
Yellow corn-----	27. 5	Dried yeast-----	4. 0
Oatmeal-----	20. 0	Calcium carbonate-----	4. 0
Wheat-----	25. 0	Sodium chloride-----	0. 5
Dried beef-----	15. 0		
Dried egg or egg yolk-----	4. 0		100. 0

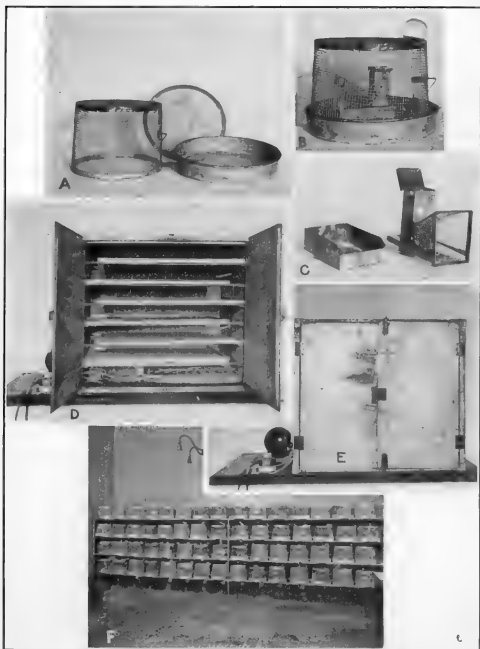
In addition to this ration the rats are given fine-cut cabbage at frequent intervals and water.

SYSTEM OF TESTING FOR VITAMIN A

Young rats weighing approximately 40 grams each and not exceeding 30 days in age, when they reach that weight are selected for the tests. As a rule, four rats are fed each ration. No attempt is made to select rats from different litters, and the sexes are ordinarily about equally divided. Each rat is weighed at the beginning of the test and at regular semiweekly intervals thereafter for a period of 90 days, unless the experiment is terminated earlier by nutritive failure or by some untoward circumstance.

Each rat is kept in an individual, cylindrical-shaped cage with a separate pan bottom, as shown in Plate 1, A and B. The dimensions of the cage are as follows: The upper part is 10¼ inches in diameter at the top, 11¼ inches at the bottom, and 8 inches high. The heavy clip projecting midway on the outside prevents the cages sticking together when stacked. A hole in the top allows insertion of the glass drinking vessel, which is held in place by a removable spring clip on the inside of the cage. The pan bottom is 11¾ inches in diameter at the bottom, 12½ inches at the top, and 2¼ inches deep. The slight flare to the sides permits stacking. A circular screen made of ¼-inch mesh galvanized wire, and slightly smaller than the pan, is used to protect a circular sheet of heavy blotting paper which serves as an absorbent.

Each cage is provided with a self-feeder (3) as shown in Plate 1, C, which holds approximately 100 grams of feed. By this means an accurate record is kept of the feed consumed.



- A.—Cage used for housing individual experimental rats, showing parts
- B.—Rat cage set up ready for use. The self-feeder and drinking vessel have been placed in the cage
- C.—Self-feeder for rats showing parts
- D.—Drying oven with doors open showing pans for holding meat or casein and electro-heating units on the bottom of the oven
- E.—Oven used for drying meat and for drying casein. The inside dimensions are 30 by 27 by 20 inches. The walls and doors are 1 inch thick and are filled with asbestos. The oven is made of galvanized iron. The blower shown at the left has a $2\frac{1}{4}$ -inch outlet and is run by a one-tenth horsepower motor. The air outlet is at the upper right end of the oven and lower down are shown thermometers. The switch in front of the blower controls the heating units in the bottom of the oven
- F.—View in animal laboratory of the biochemic division showing vitamin feeding tests in progress. This stack of shelves accommodates 128 cages and a like number of rats

As a routine procedure each rat on test is cared for daily, except Sunday, as follows: The self-feeder is examined and any feed that has been pulled into the catch pan or base is returned to the hopper, as well as any feed that has been scattered on the bottom of the cage. When nearly empty the feeder is removed and weighed, and it is replaced by a clean feeder filled with feed. The blotting paper is changed daily, except Sunday, and the drinking vessels are filled as often as necessary. The cages, feeders, and drinking vessels are cleaned and sterilized once a week.

A complete record for each rat is kept. Growth curves for the several rats that are fed the same ration are platted on one sheet. All rations tested are numbered serially.

Unless otherwise noted, the rations fed in the vitamin A tests were made up according to the following standard:

Standard ration

	Per cent
Protein (N x 6.25)-----	20
Fat-----	10
Ash mixture-----	4
Dried yeast-----	10
Starch, etc-----	56
	<hr/> 100

The 20 per cent of protein comprises that present in the dried meat which is being tested for vitamin A plus sufficient protein from dried vitamin-A-free muscle tissue or casem to make up the difference, the yeast protein not being included. The 10 per cent of fat is the sum of the fat in the dried meat and sufficient hardened cottonseed oil to make the required amount. The ash mixture is made up according to a formula of Drummond and Watson (1, p. 237). Dried baker's yeast and cassava starch comprise the balance of the ration. The proportion of protein in the ration, as well as that of dried yeast, is considerably higher than is necessary for normal growth; but this is a distinct advantage, since it guards against a deficiency of either protein or vitamin B becoming a limiting factor in the growth of the rats in case of reduced food intake due to a deficiency of vitamin A. In some rations in which relatively large proportions of dried meat were incorporated the percentage of protein, as well as that of fat, frequently exceeded the proportion stated in the standard ration.

Ash mixture

	Grams		Grams
Sodium chloride-----	5. 2	Calcium superphosphate-----	16. 2
Magnesium sulphate-----	8. 0	Calcium lactate-----	39. 0
Sodium dihydrogen phosphate-----	10. 4	Ferric citrate-----	3. 5
Dipotassium hydrogen phosphate-----	28. 6	Potassium iodide-----	Trace.
		Manganese sulphate-----	Trace.

PURIFICATION OF INGREDIENTS IN RATION

The greatest difficulty encountered in the estimation of the vitamin A content of a food product is the preparation of a basal ration free from vitamin A or containing a very small amount of it. After considerable experimentation the desired result has been accomplished. It was found that the cassava starch, dried yeast, and hardened cottonseed oil contained no vitamin A and so did not need purification.

In the earlier work reported in this paper, both the starch and the yeast were thoroughly extracted with ether before being used, but this practice was discontinued as soon as it was found to be unnecessary. Both the crude casein and the dried ox muscle used as sources of protein were found to contain considerable vitamin A and purification was required. Purified ox muscle was used in most of the tests reported in this paper, but later it was found more satisfactory to purify casein, and that product was then used exclusively as the supplementary source of protein in these vitamin A studies.

The ox muscle was purified as follows: Water-insoluble muscle tissue, a by-product resulting from the preparation of beef broth for the growth of tubercle bacilli, was dried, ground fine, and extracted with ether in large percolators until the material was practically free from fat. The extracted muscle was mixed with 60 per cent alcohol by weight in a large dish, transferred to large percolators, and extracted with 60 per cent alcohol until the extract was of a very light straw color. It was found to be practically impossible to obtain a colorless extract. The extraction with ether required two or three days and that with alcohol four or five days. The extracted material was dried and tested for vitamin A, but was found still to contain an appreciable amount of the vitamin. In order to reduce the vitamin content still further, the extracted muscle tissue was then heated in a current of air at an average temperature of approximately 115° C. for 24 hours, when it was found to be practically free from vitamin A. This method was found to be very tedious and later was abandoned.

The following method has been used regularly since October, 1923, in the purification of commercial casein, with very satisfactory results. Six hundred grams of granular casein is spread out in a thin layer in a shallow, galvanized-iron pan 18½ by 25½ inches by ½ inch deep, and five of these pans are set at one time in the drying oven shown in Plate 1, D and E. The casein is then heated in a current of air at an average temperature of 115° C. for 24 hours. The pans are then taken out and the casein is transferred to a large dish, thoroughly mixed, and returned to the pans. Heating is continued for another 24 hours, when the casein is nearly always found to be free from vitamin A as determined by feeding tests with rats. If the rats make more growth than is considered normal for the rats on a vitamin-A-free ration, the casein is heated for an additional 24 hours and tested again. Ordinarily about 25 pounds of casein is purified, thoroughly mixed, and tested in advance of requirements for the product. The heating changes the color of the casein to a very light brown but does not char the product. Comparative feeding tests have shown that rats fed a ration containing 20 per cent of protein from the purified casein made equally as good growth as those that received a ration containing a like percentage of the untreated casein, the rations being adequate in other respects.

FEEDING TESTS WITH BASAL RATIONS

Five lots of purified ox muscle and one lot of purified casein, which comprise all of these products that were used as sources of protein in the feeding tests reported in this paper, were tested to determine their freedom from vitamin A, with the results shown in

Figure 1. The first two groups of rats, Nos. 204 and 222, respectively, made slightly more growth than the three other groups, and this is probably due to the presence of a small amount of vitamin A in the two lots of purified ox muscle fed the first two groups of rats. The growth made by the rats in the last three groups, Nos. 234, 297, and 343, represents about as good results as the writers have been able to obtain after having tested a considerable number more of highly purified basal rations. This indicates that the slight growth made by the

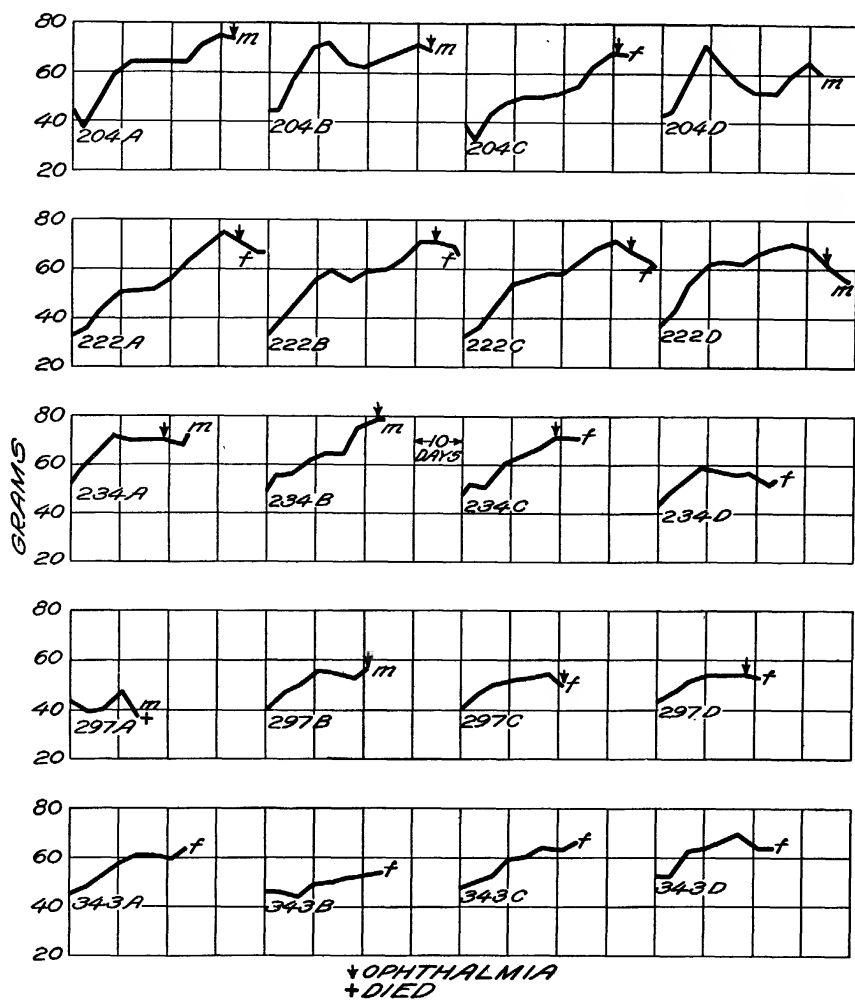


FIG. 1.—Basal rations. Graphs showing rate of growth of five groups of rats, each group being fed a basal ration practically free from vitamin A but otherwise adequate. The rations fed to the first four groups of rats, including animals Nos. 204 A to 297 D, were made up in parts by weight as follows: Ox muscle treated to remove vitamin A, to make 20 parts protein; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil sufficient to make 10 parts fat; and cassava starch to make 100. A different lot of ox muscle was used in each of the rations.

The ration fed to rats 343 A to 343 D, inclusive, was made up in the same proportions, except that casein, treated to destroy vitamin A, was substituted for ox muscle.

The sex of each rat is indicated by the letter at the end of each graph, m denoting male, f, female.

last three groups of rats shown in Figure 1 was probably due to the reserve store of vitamin A in the tissues of the young rats, and that the growth made by the first two groups of rats was due chiefly to the same factor.

Ophthalmia developed in most of the rats, as indicated, but it is not the writers' practice to continue a feeding test for any considerable time after a rat ceases to gain in weight even though ophthalmia is not evident.

VITAMIN A IN BEEF DESCRIPTION OF SAMPLES

The beef, which consisted of the rounds from the carcasses of fat steers, was purchased from local packers who slaughter their own cattle and from the branch houses of western packers. Two of the steers were slaughtered in Chicago, one in St. Louis, one in Omaha, and six in Washington, D. C. The rounds were purchased one at a time, as needed, and were prepared for use in the feeding tests in the following manner.

A cross section weighing from 20 to 25 pounds was cut from the thickest part of the round, the muscle tissue was trimmed free from fat and connective tissue, ground, mixed with water and toluol in the proportion of 800 grams of meat, 400 c. c. water and 40 c. c. toluol. The mixture was spread out in a thin layer in one of the shallow pans belonging to the drying oven shown in Plate 1, D and E. The oven was filled with five pans of the meat prepared in this manner, and the material was dried in a current of air at a temperature that did not exceed 60° C. The thin layer of meat became dry on the surface in 2 or 3 hours, when it was turned over by means of a spatula in order to expose the moist undersurface, and drying was continued overnight. The meat was thoroughly dried in from 20 to 24 hours,

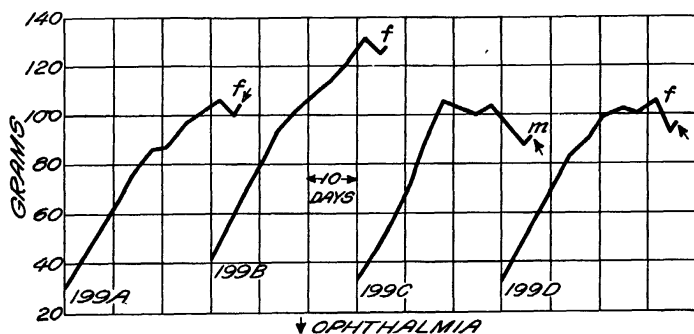


FIG. 2.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried round steak No. 869, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.9; cassava starch, 51.1; total, 100

when it was ground fine and stored in stoppered bottles in the dark at a temperature of 34° to 36° F. until needed, the feeding tests being begun as promptly as possible. Each lot of dried beef was analyzed for nitrogen and fat before being used in a ration.

FEEDING TESTS WITH BEEF

In Figure 2 are shown the growth curves of four rats that were fed a ration containing 30 per cent of dried beef No. 869 as the sole source of vitamin A in an otherwise adequate diet. The rats grew at a fair rate for a time, but growth soon ceased; 3 of the rats developed ophthalmia, and one of these had rhinitis also. It is evident that the amount of vitamin A supplied by the 30 per cent of dried beef in this ration was not nearly sufficient to meet the normal requirements of the rats.

In Figure 3 are shown the growth curves of four rats that were fed a ration containing 30 per cent of dried beef No. 874 as the sole source of vitamin A. These rats made less satisfactory growth than those shown in Figure 2. Three of the animals developed ophthalmia and two died. The amount of vitamin A supplied in this ration was far from adequate for the needs of the rats.

In Figure 4 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 902 as the sole source of vitamin A. Neither group of rats made much growth, about what might be expected from the reserve of vitamin A in their tissues, and every rat developed ophthalmia.

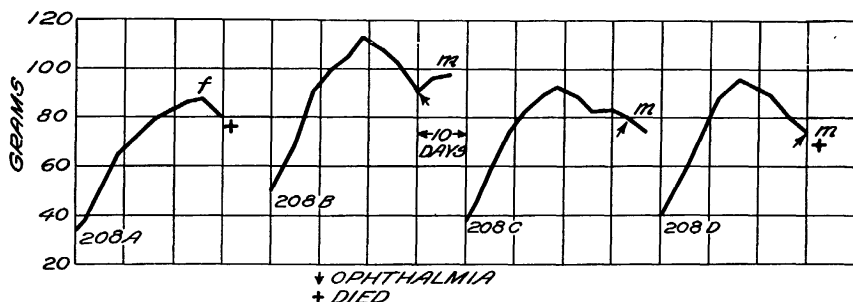


FIG. 3.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried round steak No. 874, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 5.9; cassava starch, 50.1; total, 100

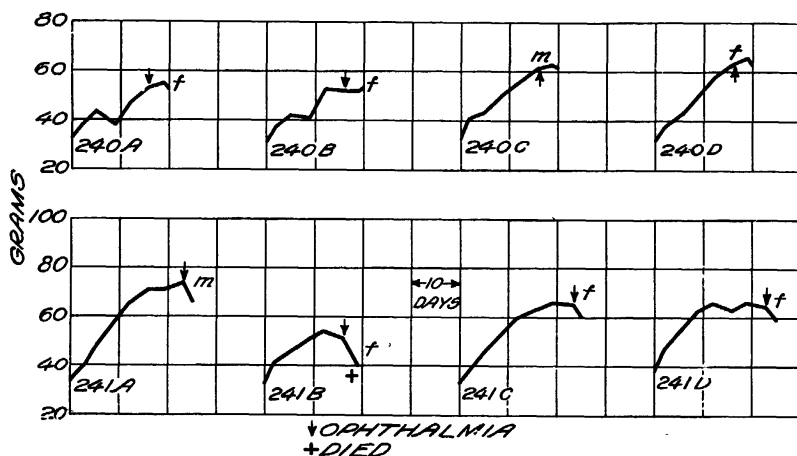


FIG. 4.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 240 A to 240 D inclusive, was made up in parts by weight as follows: Dried beef No. 902, 15; purified ox muscle, 8.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.4; cassava starch, 54.8; total 100

The ration fed to rats Nos. 241 A to 241 D, inclusive, was made up as follows: Dried beef No. 902, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.8; cassava starch, 51.2; total, 100

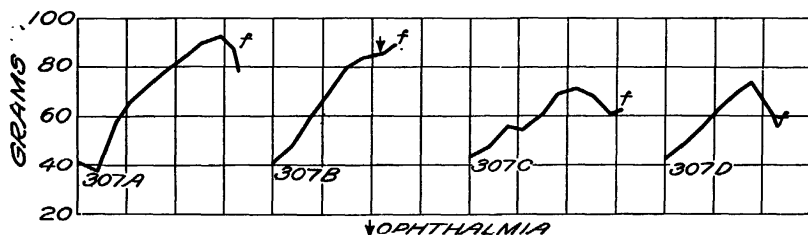


FIG. 5.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried beef No. 906, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.2; cassava starch, 52.8; total, 100

In Figure 5 are shown the growth curves of rats that were fed a ration containing 30 per cent of dried beef No. 906 as the sole source of vitamin A. Two of the rats made appreciable growth, but the other two grew about as much as rats fed one of the more satisfactory basal rations. The difference in the growth of the two pairs of rats may be due to the fact that they were from different litters.

The growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 912 as the sole source of vitamin A are shown in Figure 6. The rats getting the ration containing 30 per cent of dried beef made slightly more growth than the other group, but the amount of vitamin A in this ration was far from adequate, and every rat in the two groups developed ophthalmia.

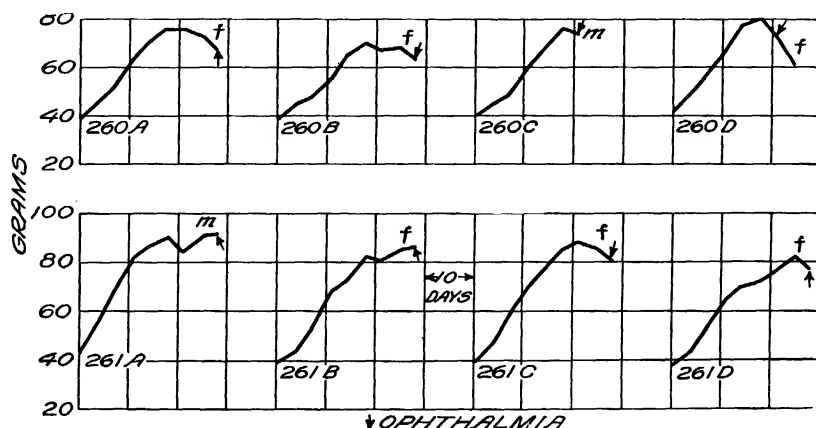


FIG. 6.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 260 A to 260 D, inclusive, was made up in parts by weight as follows: Dried beef No. 912, 15; purified ox muscle, 9.2; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.6; cassava starch, 54.2; total, 100

The ration fed to rats Nos. 261 A to 261 D, inclusive, was made up as follows: Dried beef No. 912, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 5.1; cassava starch, 50.9; total, 100

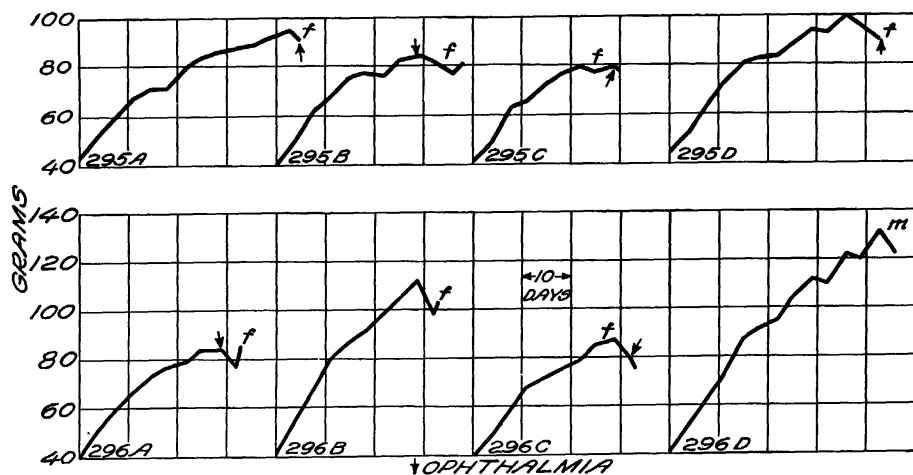


FIG. 7.—Vitamin A in beef. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried round steak as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 295 A to 295 D, inclusive, was made up in parts by weight as follows: Beef No. 934, 15; purified ox muscle, 9.4; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.2; cassava starch, 54.4; total, 100

The ration fed to rats Nos. 296 A to 296 D, inclusive, was made up as follows: Beef No. 934, 30; dried baker's yeast, 10; hardened cottonseed oil, 5.6; ash mixture, 4; cassava starch, 50.4; total, 100

In Figure 7 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried beef No. 934 as the source of vitamin A. The average rate of growth of the rats getting the ration containing 30 per cent of the dried beef is somewhat higher than that of the other group, but the growth of all the rats is far below normal. Ophthalmia developed in six of the rats.

Thus far it appears that none of the samples of dried beef tested, even when used to the extent of 30 per cent of the ration, furnished sufficient vitamin A for normal growth in young rats. In Figure 8, however, are shown the growth curves of a group of rats that received 50 per cent of dried beef Nos. 1018, 1021, and 1024 as the sole source of vitamin A and which made practically normal growth. Rat No.

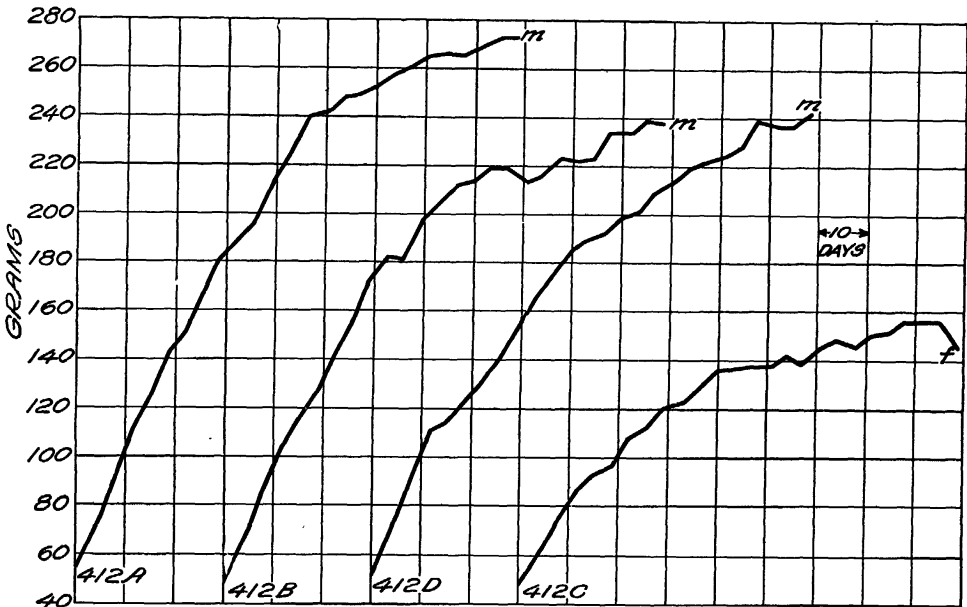


FIG. 8.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 50 per cent of dried round steak as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried beef Nos. 1018, 1021, and 1064, 50; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2; cassava starch, 34; total, 100

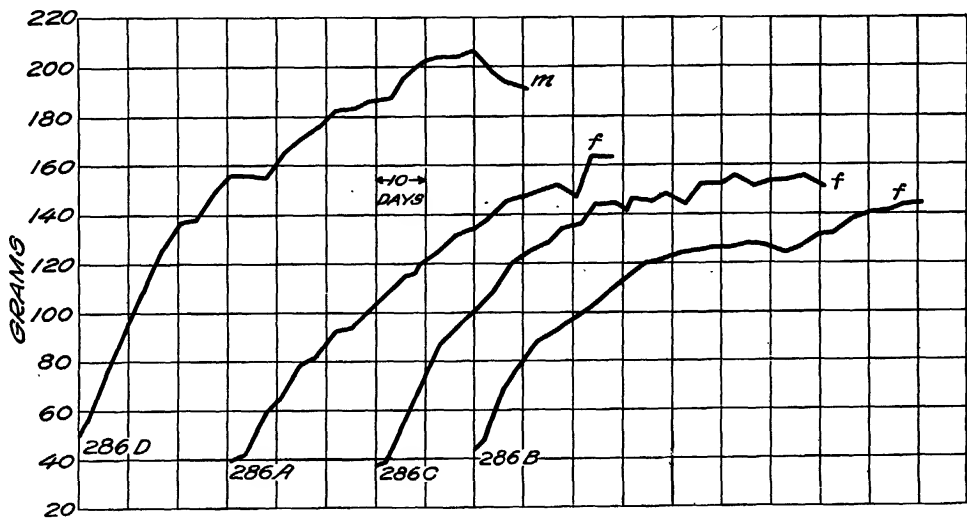


FIG. 9.—Vitamin A in beef. Graphs showing rate of growth of rats that were fed a ration containing 95 per cent of dried round steak as the source of vitamins A and B and of protein. The ration was made up in parts by weight as follows: Dried beef, 95; calcium carbonate, 4.5; sodium chloride, 0.5; total, 100

412 A, a male, made fully normal growth and was in fair condition at the end of the test. The other male rats, Nos. 412 B and 412 D, did not make quite so rapid growth and were in fair condition at the close of the experiment. The female rat, No. 412 C, did not make quite normal growth and was in fair condition at the end.

In Figure 9 are shown the growth curves of rats that were fed a ration containing 95 per cent of dried beef as the sole source of vitamins A and B and of protein. During the course of the test samples of beef Nos. 869, 874, 902, 906, 912, and 934, which also had been used in the tests already reported, and in addition sample No. 957, were used. The rats made nearly, though not quite, normal growth, but it can not be stated positively that lack of vitamin A was the limiting factor, since no vitamin B was added to the ration. All the rats were in good condition at the end of the test.

VITAMIN A IN PORK

DESCRIPTION OF SAMPLES

The pork consisted of six lots of pork loins and one lot of frozen tenderloins. Each lot of pork loins consisted of three or four loins weighing 6 to 8 pounds each. One lot had its origin in South St. Paul, one in Omaha, two in Chicago, and two in Washington, D. C. The lot of tenderloins consisted of 12 pieces weighing slightly less than 1 pound each. The origin of this product is not known. The above-described meat was purchased in Washington from local packers and from the branch houses of western packers.

The pork loins were boned out and the lean meat was separated as completely as practicable from fat and connective tissue, and was ground and dried in the manner previously described for beef. The tenderloins were trimmed free from visible fat and were dried in like manner. Each sample of dried pork was analyzed for nitrogen and fat before being used in a ration. Feeding tests were begun promptly after the meat had been dried.

FEEDING TESTS WITH DRIED PORK LOINS

In Figure 10 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried pork loin No. 873 as the sole source of vitamin A. Of the group receiving the ration that contained 15 per cent of dried pork loin, three rats made but little more growth than might have been expected on account of the store of vitamin A in their tissues, while the fourth rat made fair growth for 30 days, when it died.

Rats Nos. 206 A to 206 D, inclusive, that were fed a ration containing 30 per cent of the same lot of dried pork, made somewhat better growth than the first three rats in the preceding group, but all finally declined in weight, three developed ophthalmia, and the fourth died.

In Figure 11 are shown the growth curves of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried pork loin No. 884 as the sole source of vitamin A. Of the rats that were fed the ration containing 20 per cent of dried pork, three made fair growth but the other did not do so well. All finally declined in weight, two rats developing ophthalmia.

Rats Nos. 228 A to 228 D, inclusive, that were fed the ration containing 30 per cent of the same lot of dried pork, made somewhat better growth, but nutritive failure finally resulted. Three rats in this group developed ophthalmia and the fourth died.

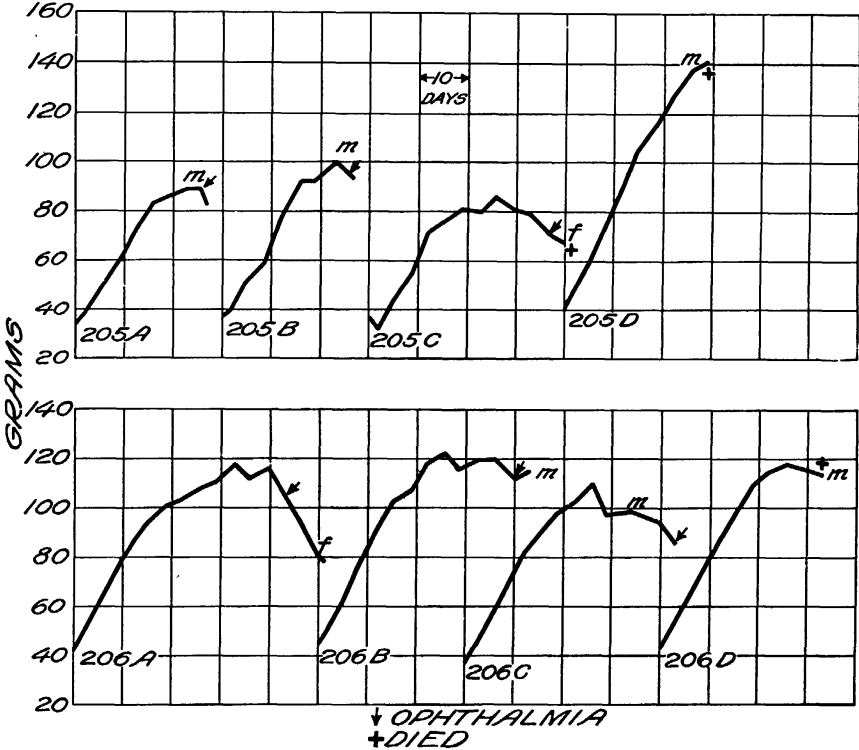


FIG. 10.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets
The ration fed to rats Nos. 205 A to 205 D, inclusive, was made up in parts by weight as follows: Dried pork loins No. 873, 15; purified ox muscle, 10.9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.9; cassava starch, 52.2; total, 100
The ration fed to rats Nos. 206 A to 206 D, inclusive, was made up as follows: Dried pork loin No. 873, 30; purified ox muscle, 1.9; ash mixture, 4; hardened cottonseed oil, 4.3; cassava starch, 59.8; total, 100

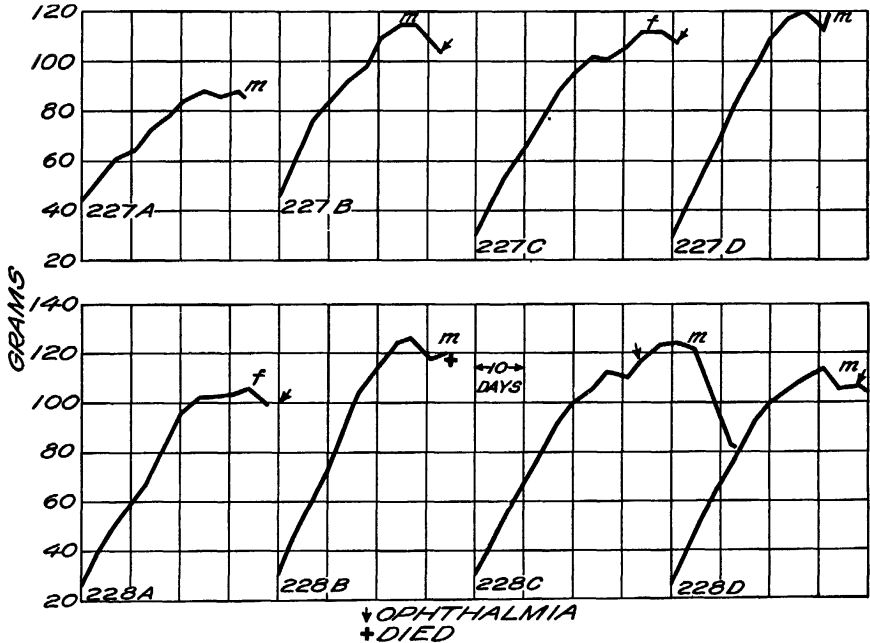


FIG. 11.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets
The ration fed to rats Nos. 227 A to 227 D, inclusive, was made up in parts by weight as follows: Dried pork loins No. 884, 20; purified ox muscle, 10.2; dried baker's yeast, 5; ash mixture, 4; hardened cottonseed oil, 1.1; cassava starch, 59.7; total, 100
The ration fed to rats Nos. 228 A to 228 D, inclusive, was made up as follows: Dried pork No. 884, 30; purified ox muscle, 5.3; dried baker's yeast, 5; ash mixture, 4; cassava starch, 55.7; total, 100

In Figure 12 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried pork loin No. 903 as the sole source of vitamin A. Rats Nos. 242 A to 242 D, inclusive, that were fed the ration containing 15 per

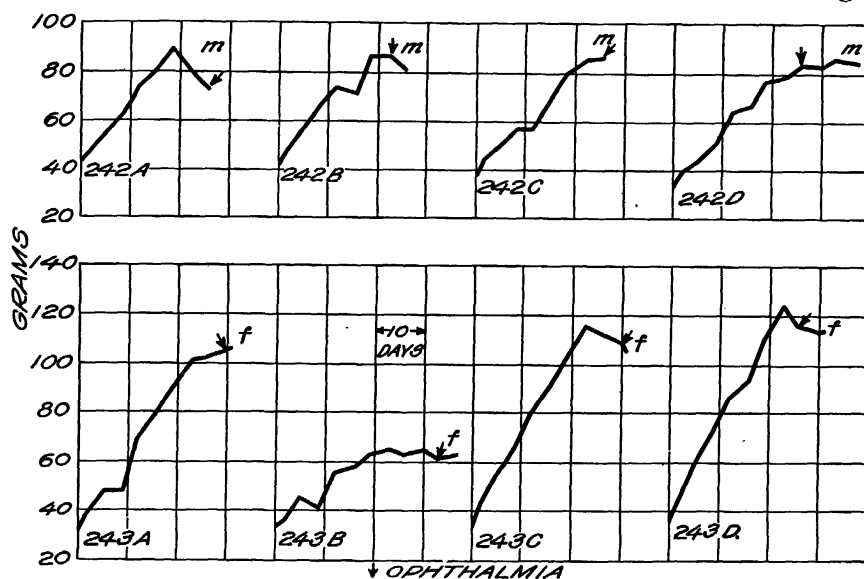


FIG. 12.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 242 A to 242 D, inclusive, was made up in parts by weight as follows: Dried pork loin No. 903, 15; purified ox muscle, 13.2; dried brewer's yeast, 5; ash mixture, 4; cassava starch, 62.8; total, 100

The ration fed to rats Nos. 243 A to 243 D, inclusive, was made up as follows: Dried pork loin No. 903, 30; purified ox muscle, 6.4; dried brewer's yeast, 5; ash mixture, 4; cassava starch, 54.6; total, 100

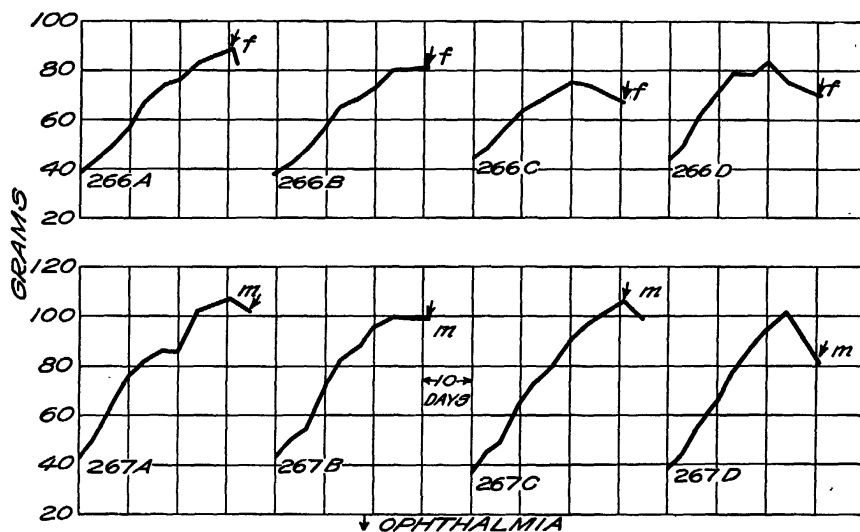


FIG. 13.—Vitamin A in pork. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 40 per cent, respectively, of the dried lean meat from pork loins as the source of vitamin A in otherwise adequate diets

The rations fed to rats Nos. 266 A to 266 D, inclusive, was made up in parts by weight as follows: Dried pork loin No. 920, 20; purified ox muscle, 5.4; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.8; cassava starch, 53.8 total, 100

The ration fed to rats Nos. 267 A to 267 D, inclusive, was made up as follows: Dried pork loin No. 920, 40; ash mixture, 4; hardened cottonseed oil, 3.6; cassava starch, 52.4; total, 100

cent of dried pork, did not make much growth and all developed ophthalmia. Three of the rats that were fed the ration containing 30 per cent of dried pork made fair growth, reaching weights between 106 and 124 grams, while the fourth made only slight growth, but all the rats in this group developed ophthalmia.

The growth curves of two groups of rats that were fed rations containing 20 and 40 per cent, respectively, of dried pork loin No. 920 as the source of vitamin A are shown in Figure 13. Rats Nos. 266 A to 266 D, inclusive, that were fed the ration containing 20 per cent of dried pork, made poor growth and all developed ophthalmia, while rats Nos. 267 A to 267 D, inclusive, that received the ration containing 40 per cent of dried pork, made slightly better growth, reaching weights between 100 and 106 grams. All the rats in the two groups developed ophthalmia by the thirty-fifth day of the test.

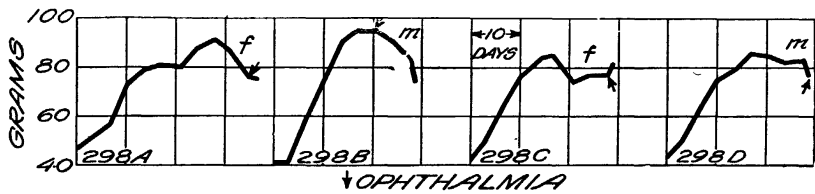


FIG. 14.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 40 per cent of dried lean meat from pork loins as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork loin No. 938, 40; ash mixture, 4; cassava starch, 56; total 100

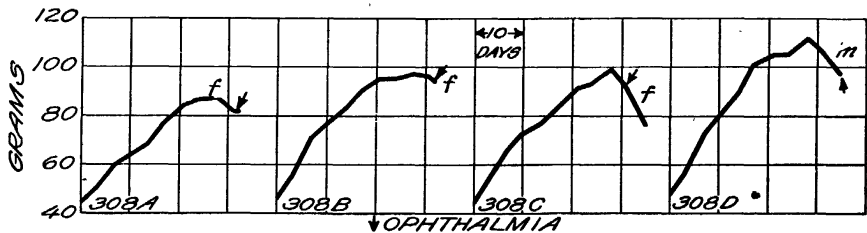


FIG. 15.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 40 per cent of dried lean meat from pork loins as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork loin No. 907, 40; ash mixture, 4; cassava starch, 56; total, 100

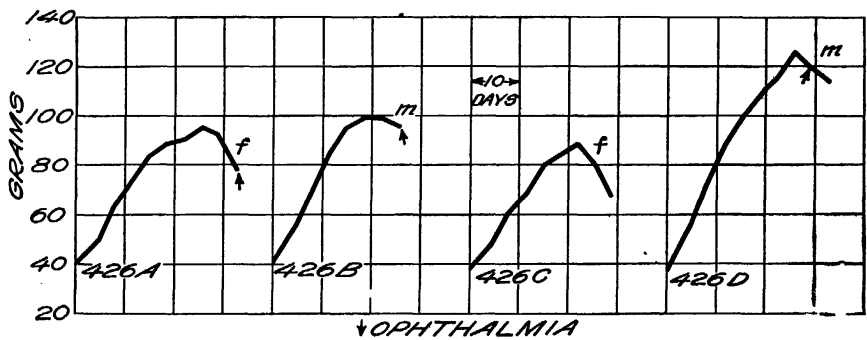


FIG. 16.—Vitamin A in pork. Graphs showing rate of growth of rats that were fed a ration containing 50 per cent of dried pork tenderloin as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried pork tenderloin No. 1024, 50; ash mixture, 4; cassava starch, 46; total, 100

In Figure 14 are shown the growth curves of rats that were fed a ration containing 40 per cent of dried pork loin No. 938 as the source of vitamin A. The rats made poor growth and all soon declined in weight and developed ophthalmia.

In Figure 15 are shown the growth curves of rats that were fed a ration containing 40 per cent of dried pork loin No. 907 as the source of vitamin A. These rats made only slightly better growth than those shown in Figure 14 and all soon declined in weight and developed ophthalmia.

In Figure 16 are shown the growth curves of rats that were fed a ration containing 50 per cent of dried pork tenderloin No. 1024 as the sole source of vitamin A. Three of the rats made poor growth, while the fourth did somewhat better, but all finally declined in weight, and three of the rats developed ophthalmia.

In Figure 17 are shown the growth curves of rats that were fed a ration consisting of 95 per cent dried pork loins, 4.5 per cent calcium carbonate, and 0.5 per cent sodium chloride. Three lots of pork loins Nos. 873, 884, and 903, that had been used in experiments already reported, were fed during the course of the experiment. While this group of rats made much better growth than any of the other groups that were fed rations containing dried pork as a source of vitamin A, they did not make nearly normal growth and all developed ophthalmia.

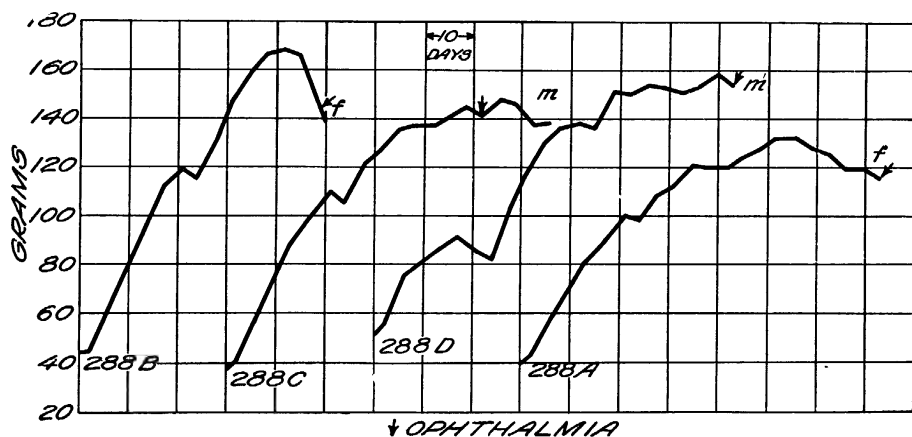


FIG. 17.—Vitamin B in pork. Graphs showing rate of growth of rats that were fed a ration containing 95 per cent of dried lean meat from pork loins as the source of vitamins A and B and of protein. The ration was made up in parts by weight as follows: Dried pork loins, samples Nos. 873, 884, and 903, 95; calcium carbonate, 4.5; sodium chloride, 0.5; total, 100

VITAMIN A IN LAMB

DESCRIPTION OF SAMPLES

Six samples of lamb were tested for vitamin A. Each of five samples was prepared from the hind saddle of the carcass of high-grade, heavy, fat lamb, while the other sample was prepared from two hind saddles of lighter weight fat carcasses. The hind saddles of the heavy lambs weighed from 18 to 20 pounds each, while the lighter saddles weighed 12 pounds each. One lamb carcass had its origin in East St. Louis, Ill., one in Omaha, Nebr., one in South St. Joseph, Mo., and the other three lambs were slaughtered at Benning, D. C. The muscle tissue was separated from fat and connective tissue and dried in the manner previously described for beef. Each sample of dried lamb was analyzed for nitrogen and fat before being incorporated in a ration.

FEEDING TESTS WITH LAMB

In Figure 18 are shown the growth curves of rats that were fed a ration containing 30 per cent of dried lamb No. 870 as the sole source of vitamin A in an otherwise adequate diet. Each of the rats made very good growth for a time, but all finally ceased to gain, then lost weight, and developed ophthalmia. One female rat, No. 201 C, nearly reached normal weight and then declined and developed oph-

thalmia. This sample of dried lamb is richer in vitamin A than any of the samples of dried beef or pork which have been reported in this paper.

In Figure 19 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb No. 876 as the source of vitamin A. Neither group of rats made much growth, all finally declined in weight, and six of the eight rats developed ophthalmia.

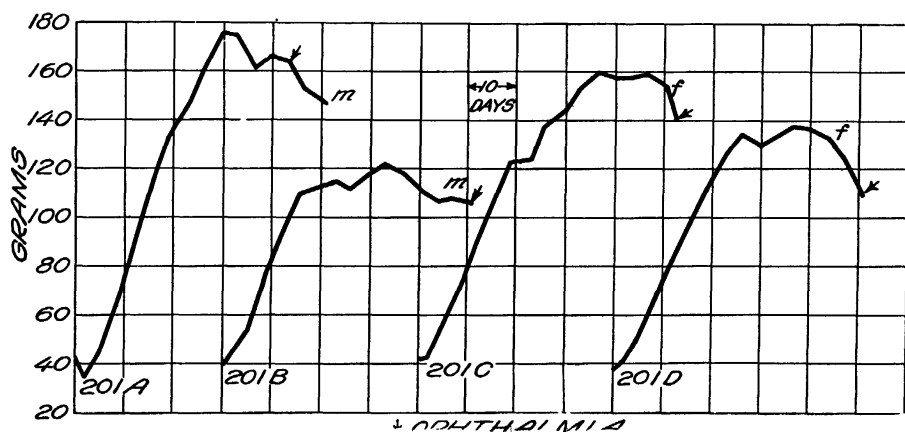


FIG. 18.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 30 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 870, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.5; cassava starch, 52.5; total, 100

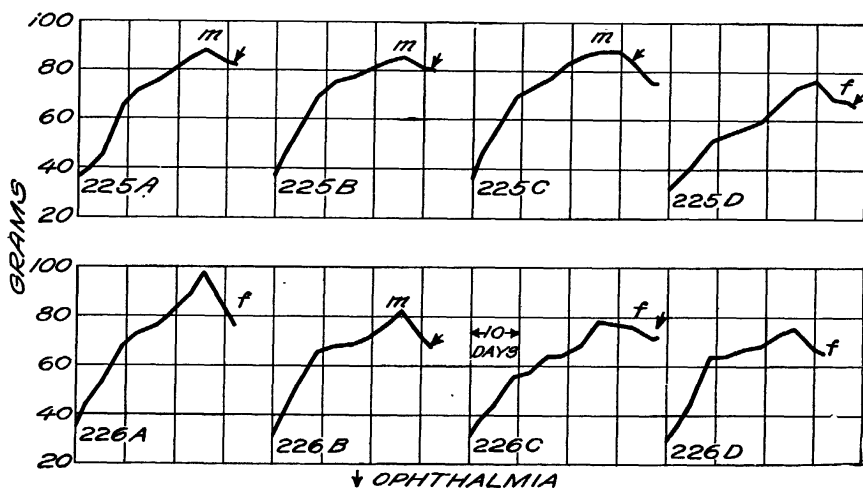


FIG. 19.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb as the source of vitamin A in an otherwise adequate diet

The ration fed to rats Nos. 225 A to 225 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 876, 15; purified ox muscle, 10.5; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.1; cassava starch, 54.4; total, 100

The ration fed to rats Nos. 226 A to 226 D, inclusive, was made up as follows: Dried lamb No. 876, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2.1; cassava starch, 53.9; total, 100

The growth curves of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried lamb No. 897 are shown in Figure 20. Both groups of rats made poor growth, only little if any better than that made by rats fed a basal ration free from vitamin A. Every rat developed ophthalmia in 22 days or less.

In Figure 21 are shown the growth curves of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb No. 933 as the source of vitamin A. With the exception

of one rat, No. 293 B, which made only fair growth and then declined in weight, the rats that were fed these rations made very poor growth. Six of the eight rats developed ophthalmia.

In Figure 22 are shown the growth curves of rats that were fed a ration containing 20 per cent of dried lamb No. 992 as the source of vitamin A. All the rats made excellent growth, practically

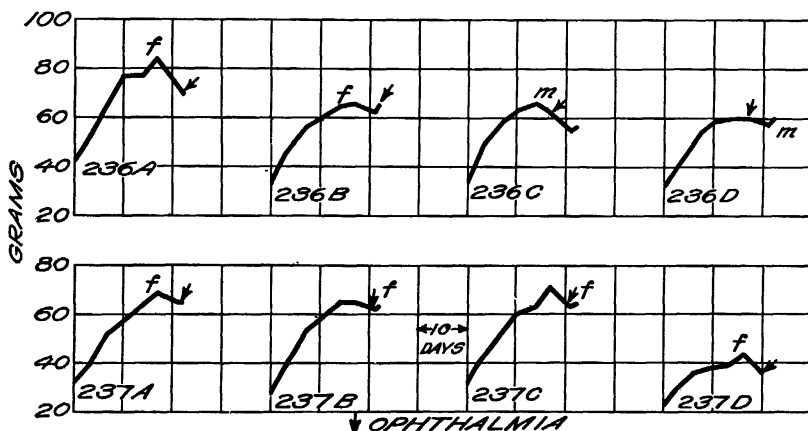


FIG. 20.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 20 and 30 per cent, respectively, of dried lamb as the source of vitamin A in otherwise adequate diets

The ration fed to rats Nos. 236 A to 236 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 897, 20; purified ox muscle, 7.9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 4.6; cassava starch, 53.5; total, 100

The ration fed to rats Nos. 237 A to 237 D, inclusive, was made up as follows: Dried lamb No. 897, 30; purified ox muscle, 1.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 2; cassava starch, 52.2; total, 100

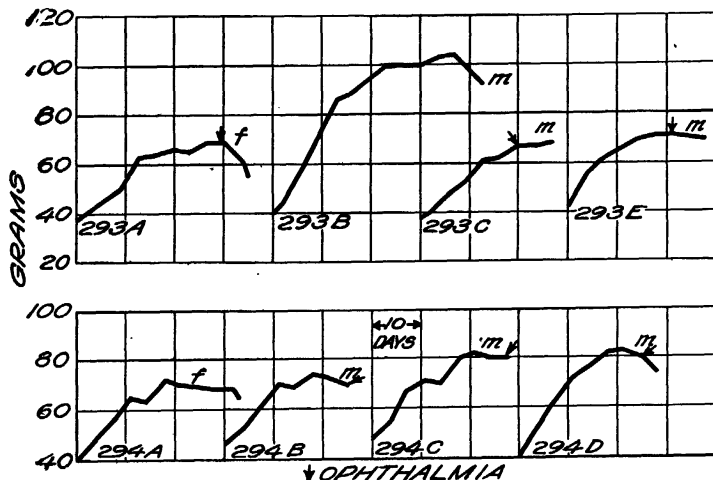


FIG. 21.—Vitamin A in lamb. Graphs showing rate of growth of two groups of rats that were fed rations containing 15 and 30 per cent, respectively, of dried lamb as the source of vitamin A in otherwise adequate diets.

The ration fed to rats Nos. 293 A to 293 E, inclusive, was made up in parts by weight as follows: Dried lamb No. 933, 15; purified ox muscle, 9; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 7.7; cassava starch, 54.3; total, 100

The ration fed to rats Nos. 294 A to 294 D, inclusive, was made up in parts by weight as follows: Dried lamb No. 933, 30; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 6.6; cassava starch, 49.4; total, 100

normal for the sexes. One male, No. 372 C, reached the maximum weight for the group and then rapidly declined in weight to 181 grams when it was removed from the test. This rat was in very poor condition at the time but showed no signs of ophthalmia. The three other rats gained in weight until the close of the test and were in excellent condition at the end. This sample of dried lamb is richer in vitamin A than any of the other samples of meat thus far reported in this paper.

In Figure 23 are shown the growth curves of rats that were fed a ration containing 20 per cent of dried lamb No. 994 as the source of vitamin A. The two male rats in the group made remarkably good

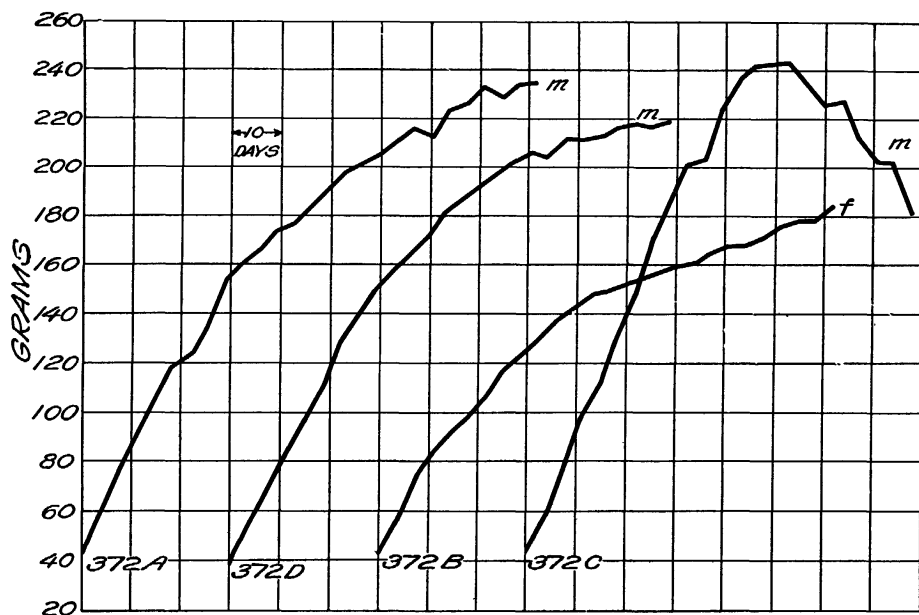


FIG. 22.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 20 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 992, 20; purified casein, 10.6; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3; cassava starch, 52.4; total, 100

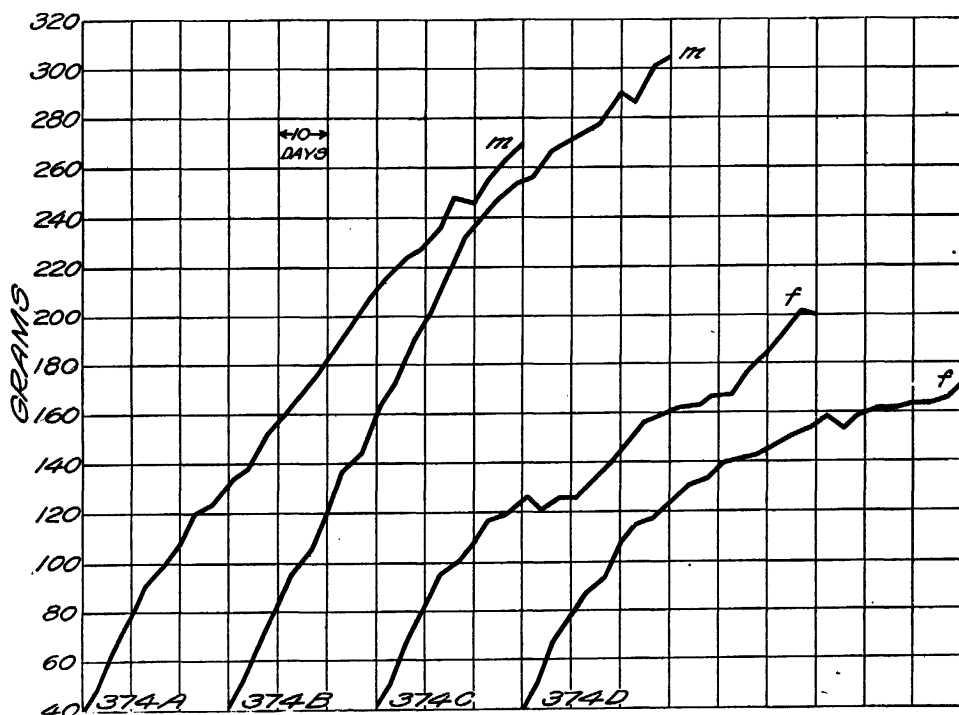


FIG. 23.—Vitamin A in lamb. Graphs showing rate of growth of rats that were fed a ration containing 20 per cent of dried lamb as the source of vitamin A in an otherwise adequate diet. The ration was made up in parts by weight as follows: Dried lamb No. 994, 20; purified casein, 9.8; dried baker's yeast, 10; ash mixture, 4; hardened cottonseed oil, 3.3; cassava starch, 52.9; total, 100

growth, while the two females did nearly as well, sex considered. With one exception, the rats were still gaining in weight when the test was discontinued at the end of 90 days, and all were in excellent condition at that time.

This sample of dried lamb is even richer in vitamin A than the preceding sample (No. 992, fig. 22). It is interesting to record that the two hind saddles of lamb from which samples Nos. 992 and 994 were prepared were purchased at the same time from a local meat-packing establishment. On inquiry it was found that the two lamb carcasses probably came out of a carload of lambs purchased in Chicago and slaughtered at Benning, D. C. No information was available as to how the lambs had been fed.

SUMMARY OF RESULTS AND CONCLUSIONS

Since the rats fed the basal rations alone made a certain amount of growth for a time, due, no doubt, principally to the store of vitamin A in their tissues, in interpreting the results of any experiment allowance must be made for the growth made by rats fed the basal ration. Unfortunately, as yet, a satisfactory unit for measuring the vitamin A content of a food product is lacking, so that the values obtained are reported only in very general terms. Since the energy values of the different rations are practically the same, the growth value obtained for one ration may be compared directly with that obtained from another.

VITAMIN A IN BEEF

A total of 10 samples of beef, representing a like number of cattle, were tested for their vitamin A content by means of feeding tests with 11 groups of rats of 4 members each, or a total of 44 rats. Six samples of dried beef Nos. 869, 874, 902, 906, 912, and 934 were used in the proportions either of 15 or 30 per cent in rations fed to 9 groups of rats. The results of these tests show that none of those samples of dried beef, in the quantities fed, furnished sufficient vitamin A to induce normal growth in young rats. The best growth was made by rats fed rations containing 30 per cent, respectively, of samples of beef Nos. 869, 874, and 934, as shown in Figures 2, 3, and 7.

When the proportion of dried meat in the ration was increased to 50 per cent, much more satisfactory results were obtained. A group of four rats was fed a ration containing 50 per cent of dried beef, samples Nos. 1018, 1021, and 1024 being used in succession. One male rat made excellent growth, two other male rats did nearly as well, and a female rat made nearly normal growth (fig. 8). All four rats were in fair condition at the close of the test. Apparently 50 per cent of these samples of dried beef in a ration furnished nearly sufficient vitamin A for normal growth.

Another group of rats was fed a ration containing 95 per cent of dried beef, the same samples that were used in the tests reported in Figures 2 to 7, inclusive, being used in succession; and, in addition, another lot of beef, No. 957 (fig. 9). The rats in this group, one male and three females, made nearly, but not quite, normal growth, and all were in good condition at the end of test.

The results of these tests show that the samples of beef tested were relatively poor in vitamin A, but that when used in rations in proportions of 50 or 95 per cent, nearly sufficient vitamin A for normal growth was furnished.

VITAMIN A IN PORK

Six lots of pork loins, representing 18 hogs, and 1 lot of pork tenderloins, representing 12 hogs, were tested for their content of vitamin A by means of feeding tests with 12 groups of 4 rats each, or a total of 48 rats. In general, the results obtained with dried fresh pork as a source of vitamin A are less satisfactory than those obtained with beef. The rations tested contained dried pork in percentages ranging from 15 to 95 per cent, but in no instance did a ration furnish sufficient vitamin A to meet the requirements of growing rats. Fair growth was made by a number of rats that were fed rations containing 30 to 40 per cent of dried pork (figs. 10, 11, and 12) and by a few rats receiving a ration containing 20 per cent of dried pork (fig. 11), but even 50 per cent of dried pork tenderloin in a ration proved to be inadequate as a source of vitamin A (fig. 16). A ration containing 95 per cent of dried pork loins did not furnish sufficient vitamin A to induce normal growth in young rats (fig. 17). Four rats were fed this ration, and, although they grew fairly well for a time, all finally declined in weight and developed ophthalmia.

The results of the experiments with fresh pork show that the samples tested were relatively poor in vitamin A.

VITAMIN A IN LAMB

A total of 6 samples of lamb, representing 7 animals, were tested for their vitamin A content by means of feeding tests with 9 groups of rats of 4 members each, or a total of 36 rats. There were rather wide differences in the vitamin A content of the several samples of lamb. Samples Nos. 876, 897, and 933 were poorer in vitamin A than the others. Each of these samples was used in the proportions of 15 and 30 per cent in rations, but none of the rats made much growth (figs. 19, 20, 21).

Sample of lamb No. 870 was somewhat richer in vitamin A, and rats that were fed a ration containing 30 per cent of this sample made considerable growth, although they finally declined in weight and developed ophthalmia (fig. 18). One male rat reached a maximum weight of 176 grams in 30 days, and a female a maximum weight of 160 grams in 37 days.

Samples of lamb No. 992 and 994 were the richest in vitamin A of any of the samples of beef, pork, or lamb that are reported in this paper. The four rats fed the ration containing 20 per cent of sample No. 992 made normal growth, and three of the rats were still gaining in weight at the end of the 90-day test, but the fourth rat, a male, declined rapidly in weight after having reached a maximum weight of 243 grams in 53 days (fig. 22). The rats that were fed a ration containing 20 per cent of the other sample of lamb, No. 994, made even better growth. The two male rats in this group attained weights of 268 and 304 grams, respectively, in 90 days, while the females weighed 171 and 200 grams, respectively, at the end of the same period (fig. 23).

The results of the vitamin A tests with lamb show that three of the six samples examined were rather poor in vitamin A, one contained a fair proportion, while the remaining two samples were richer in vitamin A than any of the other samples of meat that are reported in this paper.

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THE RELATIONSHIP BETWEEN THE WEIGHT OF EGGS AND THE WEIGHT OF CHICKS ACCORDING TO SEX¹

By M. A. JULL, *Poultry Husbandman*, and J. P. QUINN, *Chief Scientific Aid, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In the domestic fowl the relationship between the weight of eggs and the weight of chicks according to sex at hatching time is an interesting problem, inasmuch as the mature male is normally heavier than the mature female. If the chicks of both sexes weigh practically the same at hatching time, then there remains an interesting study of different rates of growth of the sexes. On the other hand, if the weights of the sexes are significantly different at hatching time, it is of importance, then, to try to discover why the weights are different and whether the sexes of standard-bred chicks can be separated according to differences in weight.

This study was undertaken to determine, first, the relationship between the weights of chicks of the two sexes at hatching time; and, second, the relationship between the weights of the eggs and the weights of the chicks hatched from them.

PROCEDURE

The eggs used in this study were obtained from four different sources: 53 Rhode Island Red yearling hens, 30 Rhode Island Red pullets, 50 Barred Plymouth Rock yearling hens, and 113 Barred Plymouth Rock pullets. All the females were mated to Rhode Island Red cockerels. The eggs were saved from March 15 to March 23, when the incubators were set. Only a portion of the Rhode Island Red eggs laid during the period mentioned were used in this study. All the eggs laid by the Barred Plymouth Rocks were used. In this paper no account is taken of the infertile eggs, embryos which died during the period of incubation, and chicks which died in shell at hatching time. The eggs were weighed daily as laid, the weights being recorded to hundredths of a gram. The chicks were weighed at hatching time, the weights also being recorded to hundredths of a gram.

The sex of the chicks from the Barred Plymouth Rock females was recorded at hatching time, and that of the Rhode Island Red chicks when the chicks were 9 weeks old. Distinguishing the sex of chicks from the Barred Plymouth Rock females mated to Rhode Island Red males was an easy matter, since the sex-linked barring pattern of the barred females is transmitted to the sons only. The male chicks always have the white spot on the top of the head and yellow shanks, characteristic of purebred Barred Plymouth Rock male chicks, while the female chicks of this cross lack the white spot and have black or very dark shanks.

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EXPERIMENTAL DATA

In Table I is shown, for each of the four groups of birds, the relationship between the weight of eggs from which males were hatched and the weight of the eggs from which females were hatched. In the case of the eggs from the Barred Plymouth Rock yearling hens, those giving rise to males have a slightly lower mean weight than the eggs giving rise to females, but in the other three cases the reverse is true. In no case, however, is the difference in weights significant, the differences with their probable errors being 0.34 ± 0.55 , 0.60 ± 0.42 , 0.44 ± 0.48 , and 0.48 ± 0.34 gm.

This situation is in harmony with the results obtained by the senior writer² in the case of eggs from 30 Barred Plymouth Rock pullets mated to Brown Leghorn males, and by both authors³ in the case of eggs from 153 Barred Plymouth Rock females mated to Rhode Island Red males. It seems, then, that selection of hatching eggs according to weight can not be expected to affect the sex ratio of chicks hatched therefrom.

TABLE I.—*Relationship between the mean weight of eggs producing males and the mean weight of eggs producing females in each of four groups; and the relationship of the mean weight of eggs producing males and females, respectively, between the yearling hen and pullet groups of each breed*

Group	Number	Mean weight of eggs from which males were hatched	Number	Mean weight of eggs from which females were hatched	Difference
Rhode Island Red yearlings.....	66	Gm. 59.88±0.36	49	Gm. 59.54±0.41	0.34±0.55
Rhode Island Red pullets.....	59	56.61±.30	54	56.01±.30	.60±.42
Difference.....		3.27±.47		3.53±.51	
Barred Plymouth Rock yearlings.....	63	59.91±.30	49	60.35±.37	.44±.48
Barred Plymouth Rock pullets.....	123	57.93±.24	106	57.45±.24	.48±.34
Difference.....		1.98±.38		2.90±.44	

Table I shows, for each of the two breeds, in the case of eggs giving rise to males and females, respectively, the difference in weights of eggs from yearling hens and from pullets. In both breeds the eggs from yearling hens were considerably heavier than the eggs from pullets. In the Rhode Island Reds, the difference in the weights of eggs giving rise to males is 3.27 ± 0.47 gm. in favor of the eggs from the yearling hens, and the difference in the weights of eggs giving rise to females is 3.53 ± 0.51 gm. in favor of the yearling hens. In the Barred Plymouth Rocks, the difference in the weights of eggs giving rise to males is 1.98 ± 0.38 gm. in favor of the yearling hens, and the difference in the weights of eggs giving rise to females is 2.90 ± 0.44 gm. In each case, the difference is considerably more than three times its probable error, and it is seen, then, that in both breeds the yearling hens laid significantly heavier eggs than the pullets.

The weights of the chicks of each sex from each of the four groups of birds are shown in Table II. In each of the four groups the mean weight of the females is lighter than that of the males, but in no

² JULL, M. A. THE RELATION OF ANTECEDENT EGG PRODUCTION TO THE SEX RATIO OF THE DOMESTIC FOWL. Jour. Agr. Research 28: 199-224. 1924.
³ JULL, M. A., and QUINN, J. P. THE SHAPE AND WEIGHT OF EGGS IN RELATION TO THE SEX OF CHICKS IN THE DOMESTIC FOWL. Jour. Agr. Research 29: 195-202. 1924.

case is the difference significant, the differences with their probable errors being 0.63 ± 0.43 , 0.81 ± 0.32 , 0.11 ± 0.43 , and 0.68 ± 0.25 gm. These results would indicate that chicks can not be assorted by sex at hatching time according to their weights.

TABLE II.—*Relationship between the mean weight of male chicks and the mean weight of female chicks, in each of four groups; and the relationship of the mean weight of male and female chicks, respectively, between the yearling hen and the pullet groups of each breed*

Group	Number	Mean weight of male chicks	Number	Mean weight of female chicks	Difference
		Gm.		Gm.	
Rhode Island Red yearlings.....	66	39.72 ± 0.31	49	39.09 ± 0.30	0.63 ± 0.43
Rhode Island Red pullets.....	59	$37.68 \pm .23$	54	$36.87 \pm .23$	$.81 \pm .32$
Difference.....		$2.04 \pm .39$		$2.22 \pm .38$	
Barred Plymouth Rock yearlings.....	63	$39.07 \pm .28$	49	$38.96 \pm .33$	$.11 \pm .43$
Barred Plymouth Rock pullets.....	123	$37.99 \pm .18$	106	$37.31 \pm .18$	$.68 \pm .25$
Difference.....		$1.08 \pm .33$		$1.65 \pm .38$	

Table II also shows the relationship between the weights of males and females, respectively, obtained from eggs laid by the yearling hens and from eggs laid by the pullets for each of the two breeds. In the case of the Rhode Island Reds, the difference in the mean weight of males from eggs laid by the yearling hens and from eggs laid by pullets is 2.04 ± 0.39 gm. in favor of the yearling hens, and the difference in the mean weight of females from eggs laid by yearling hens and from eggs laid by pullets is 2.22 ± 0.38 gm. in favor of the yearling hens. In the case of the Barred Plymouth Rocks, the difference in the mean weight of males from eggs laid by the yearling hens and from eggs laid by the pullets is 1.08 ± 0.33 gm., and the difference in the mean weight of females from eggs laid by yearling hens and from eggs laid by pullets is 1.65 ± 0.38 gm. The differences are significant in every case, and are what would naturally be expected in view of the significant differences in the mean weights of eggs laid by the yearling hens and by the pullets, as shown in Table I.

It might be possible, however, for chicks from eggs laid by pullets to be as heavy or even heavier than chicks from eggs laid by yearling hens, if the chicks from pullet eggs constitute a higher mean percentage of the mean egg weight than the chicks from yearling-hen eggs. Such a situation might be true whether the mean weight of the pullet eggs was as great or even less, as in the case of this study, than the mean weight of the yearling-hen eggs. In other words, do all chicks, whether they come from eggs laid by yearling hens or from eggs laid by pullets tend to constitute the same percentage of the weight of the eggs from which they were hatched?

The data in Table III show, for each of the four groups of birds and for the sexes, respectively, the chick weight times one hundred over the egg weight or the mean percentage chick weight of egg weight.

The female chicks constitute a lower mean percentage of the eggs from which they were hatched in all four cases. In no case, however, is the difference in mean percentage significant, the differences with their probable errors being: 0.68 ± 0.28 , 0.73 ± 0.33 , 0.60 ± 0.35 , and 0.61 ± 0.30 . It would seem, then, that in the case of neither

yearling hens nor pullets is there any significant difference in the mean percentage male and female chick weight of egg weight.

TABLE III.—*Relationship between the mean percentage chick weight of egg weight in eggs producing males and the mean percentage chick weight of egg weight in eggs producing females, in each of four groups; and the relationship between the mean percentage chick weight of egg weight in eggs producing males and females, respectively, in each breed*

Group	Number	Mean percentage chick weight of egg weight in eggs from which males were hatched	Number	Mean percentage chick weight of egg weight in eggs from which females were hatched	Difference
Rhode Island Red yearlings.....	66	66.33±0.19	49	65.65±0.21	0.68±0.28
Rhode Island Red pullets.....	59	66.56±.25	54	65.83±.22	.73±.33
Difference.....		.23±.31		.18±.30	
Barred Plymouth Rock yearlings.....	63	65.16±.23	49	64.56±.27	.60±.35
Barred Plymouth Rock pullets.....	123	65.60±.20	106	64.99±.23	.61±.30
Difference.....		.44±.30		.43±.35	

When the mean percentage chick weight of egg weight per sex between the yearling hens and pullets is considered, it is seen that in no case was there a significant difference. In every case, the mean percentage was greater in the case of the pullets than in the case of the yearlings. This is accounted for in the fact that the mean weight of the pullet eggs was somewhat lower than the mean weight of the yearling-hen eggs. The results in Table III tend to show that pullet eggs having the same weight as yearling-hen eggs tend to produce chicks having as great a mean weight as the chicks from eggs laid by yearling hens. In other words, the weight of the chick seems to be determined, more or less, by the weight of the egg from which it was hatched. The mean weight of the chicks hatched from small eggs, whether they be from yearlings or pullets, seems to be reduced according to the extent that the small eggs are used in incubation.

CONCLUSIONS

There is no significant difference between the weight of yearling-hen eggs from which males are hatched and the weight of yearling-hen eggs from which females are hatched.

There is no significant difference between the weight of pullet eggs from which males are hatched and the weight of pullet eggs from which females are hatched.

There is no significant difference in the weight of male chicks and the weight of female chicks from eggs laid by yearling hens.

There is no significant difference in the weight of male chicks and the weight of female chicks from eggs laid by pullets.

There is no significant difference in the percentage chick weight (in either sex) of egg weight in yearling hens as compared with pullets.

If pullet eggs have a significantly lower mean weight than yearling-hen eggs, the chicks hatched from pullet eggs will also tend to have a significantly lower mean weight than the chicks hatched from yearling-hen eggs.

The separation of the sexes of chicks at hatching time on the basis of weight is unreliable.

TESTS OF DEEP-WELL TURBINE PUMPS¹

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INTRODUCTION

The deep-well turbine pump is a modification of the vertical centrifugal pump so designed as to obviate the necessity of constructing a pit where the depth to water is beyond the practical limit for installing centrifugal pumps. It is built up in vertical units or bowls, one above another. Any number of bowls may be used, the number varying according to the head to be pumped against and the discharge desired, 12 to 30 feet being allowed for each stage or bowl. Each bowl contains a runner with guide or diffusion vanes. The vertical shaft extends through all the bowls, and the runners are attached to the vertical shaft. The multiplicity of runners and bowls gives a booster effect to the pump and decreases the speed at which it would be necessary to operate it if but one runner were used. The pump is entirely submerged. It is primed automatically, and oiling of the bearing is accomplished either through an oil pipe which incloses the shafting or by means of small pipes leading from an oil supply at the surface and directly attached to the bearings. Where the shaft and bearings are inclosed in an oil pipe they are protected from wear caused by sand pumped out in the discharge water.

This type of pump is adapted to pumping conditions which involve a depth to the water level of more than 75 feet, or a variable water table below the 50-foot level. As new lands in the arid West in which the depth to water does not exceed 50 feet are rare, the practice of irrigation by pumping will inevitably call for the use of large numbers of deep-well turbine pumps, which meet all the requirements of irrigation up to the economic limit of pumping for the various crops that are grown.

PURPOSE OF TESTS

Used within the range of heads for which the deep-well turbine pump is suitable, the degree of success attending its use depends upon a proper regard for the economics of selection, installation, and operation. One of the most important factors in the economical operation of all types of pumps is their efficiency. Most operators entirely lose sight of this in the endeavor to produce the largest flow possible, regardless of the expenditure of power.

For a given head, there is a direct relation between the speeds of the pump and prime mover, respectively, which must be maintained by means of pulleys of proper sizes. Where pumps are direct-connected to motors or engines, the same factor relative to speed of pump and prime mover exists. Hence, knowing the height to which water is to be raised and the quantity to be pumped, it is possible

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to determine the size of pump needed and the speed at which it should be operated to give the most economical performance.

It was for the purpose of providing data of this kind, of which very little has hitherto been published, that the Division of Agricultural Engineering, Bureau of Public Roads, United States Department of Agriculture, conducted the tests which are reported here. The principal object was to determine the proper speed of pumps for various heads and capacities. In making the tests, a study of various types of impellers was not attempted, the aim being to obtain results which would conform with field installations.

THE PUMPS TESTED

The tests were made at the laboratory of the New Mexico Agricultural College, State College, N. Mex.² (fig. 1), under a cooperative agreement between the New Mexico Agricultural Experiment Station and the Division of Agricultural Engineering of the Bureau of Public Roads.

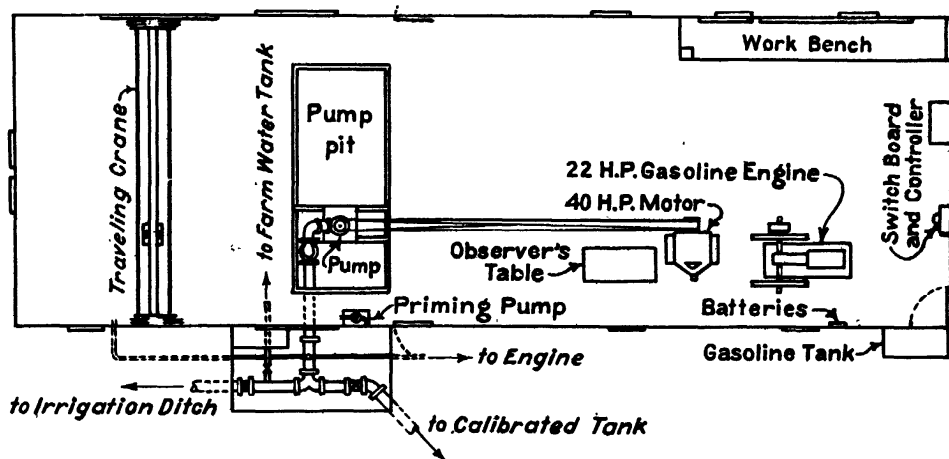


FIG. 1.—Plan of testing station

By the courtesy of three manufacturers, seven deep-well turbine pumps were obtained for testing. Pumps Nos. 1, 2, and 3 were obtained from the American Well Works, Aurora, Ill. They were 24-inch, 3-stage; 14-inch, 5-stage; and 12-inch, 7-stage, respectively. Pumps Nos. 4, 5, and 6, obtained from the Layne & Bowler Corporation of Los Angeles, were 24-inch, 2-stage; 17-inch, 4-stage; and 17-inch, 2-stage, respectively. Pump No. 7 was a 17-inch, 4-stage pump obtained from the Byron-Jackson Co. of San Francisco.

Each of these pumps was installed in a 12-inch bored and cased well with a capacity of 2,000 gallons per minute. Power was furnished by a 40-horsepower variable-speed motor with controller.

MODE OF PROCEDURE

Before the tests were started an investigation was made of the constancy of the voltage, and a prony brake test (fig. 2) was run on the motor to develop data for an efficiency curve which would con-

² Acknowledgment is due Dean W. Bloodgood, irrigation engineer, for his assistance in making pump tests, computation of data, and plotting original characteristic curves.

form with local operating conditions. Each pump was carefully installed, and all joints and connections were inspected before the tests started to insure efficient operation. The weir crest was checked for level, and the zero of the hook gauge was made to conform with the elevation of the crest of the weir.

Two men, observer and recorder, made the tests. Two sets of readings were taken separately for each observed head and these were averaged, unless there appeared to be too great a difference between readings, in which case a third set of readings was taken and the two between which there was the least difference were averaged.

The variations in head were controlled by throttling the discharge with a gate valve. Each set of tests started with the valve closed. For each succeeding run it was opened enough to give a decrement of 2 feet in head.

The cycle of readings taken by the observer was: Voltage, kilowatts, speed of motor, speed of pump, height of water on weir crest as indicated by hook gauge, and check reading on voltmeter and wattmeter. The readings taken by the recorder were: Vacuum head and pressure head, the latter being made at the time the wattmeter was read by the observer.

TEST DATA

The test data for each pump consist of tables giving complete results of the various average speeds and performance curves, including capacity-head curves, iso-efficiency curves, iso-brake-horsepower curves, and logarithmic curves showing the velocity-head difference between suction and discharge.

The tables give the speeds of the pump for varying capacities in gallons per minute and the corresponding pressure head, suction head, static head, velocity head, total head, brake horsepower, water horsepower, and efficiency. The pressure head and suction head were measured directly with mercury manometers. The static head was measured with a surveyor's level, being the distance between the taps for pressure and vacuum, respectively. Brake horsepower was measured directly by an indicating wattmeter and corrected for motor efficiency from the motor-efficiency curve. Water horsepower and efficiency were computed from the foregoing data.

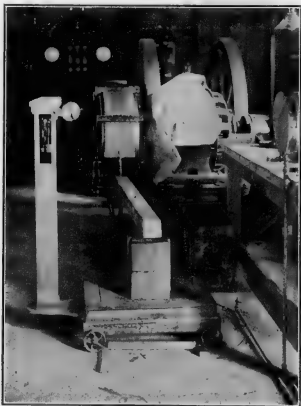


FIG. 2.—Prony brake test of motor

The velocity head is based on the net cross sectional area of discharge and suction pipes, respectively. The velocity of water in each instance was determined by this formula:

$$v = \frac{Q}{A}$$

in which v = velocity in feet per second, Q = flow in cubic feet per second, and A = net sectional area in square feet.

Velocity head was obtained from the formula:

$$h = \frac{v^2}{2g}$$

in which h = velocity head in feet, v = velocity in feet per second, and g = acceleration of gravity = 32.2.

The velocity-head difference is therefore equal to $\frac{v^2 - v_1^2}{2g}$, in

which v is the velocity of water in the discharge pipe, and v_1 is the velocity in the suction pipe. If the net area of the suction pipe at the point where the pipe is tapped for the measurement of the vacuum, is greater than that of the discharge pipe where it is tapped for the measurement of pressure, h is positive and is therefore additive to the total head composed of pressure, vacuum, and static heads. In the case of the pumps tested, the net area at the point of vacuum tap was less than the net area at the point of pressure tap, and hence the velocity of the water in the suction pipe was greater for a given volume than that in the discharge pipe. The velocity-head difference between discharge and suction pipes was therefore subtractive.

Diagrams based on the formula $h = \frac{v^2 - v_1^2}{2g}$ have been prepared

for each pump, as shown in Figures 3 to 8, inclusive, for the purpose of determining in the tests the amount of head to be added or subtracted due to the velocity of flow in the suction and discharge pipes.

In studying the diagrams, it is interesting to note the recovery of head due to velocity. Referring to Tables I and IV, comparing pumps Nos. 1 and 4, respectively, the recovery of head in pump No. 1 is shown to be 2.3 feet for a maximum flow of 1,321 gallons per minute, or 2.94 second-feet; while in pump No. 4 the recovery was 0.99 foot with a maximum flow of 1,466 gallons per minute, or 3.26 second-feet. Both pumps are 24 inches in diameter; No. 1 is 3-stage and No. 4 is 2-stage. They are of different makes, however, and the difference is due to the design, which gives a greater difference in area between discharge and suction in the case of No. 4 than No. 1. The recovery of head appears to be greater in the pumps of smaller diameter than in the larger pumps. For example, in pump No. 5, Table V, the velocity-head difference is 6.36 feet for a maximum flow of 1,092 gallons per minute, or 2.43 second-feet. This quantity is subtractive, indicating that there was no head recovered, but rather a loss of head, because the net area of discharge pipe at pressure tap was larger than the net area of the suction pipe at vacuum tap.

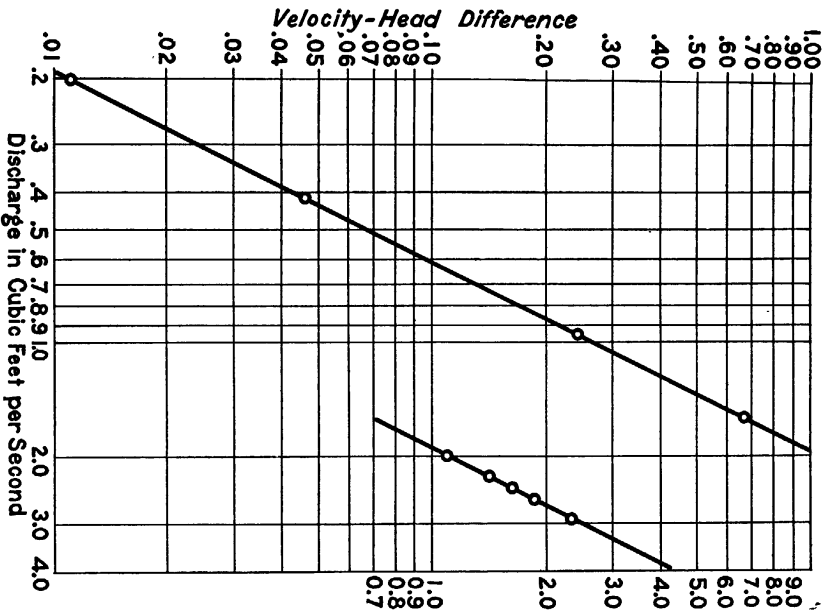


FIG. 3.—Velocity-head curve, pump No. 1, deep-well turbine pump, 3-stage, 24-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.197 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.343 square foot

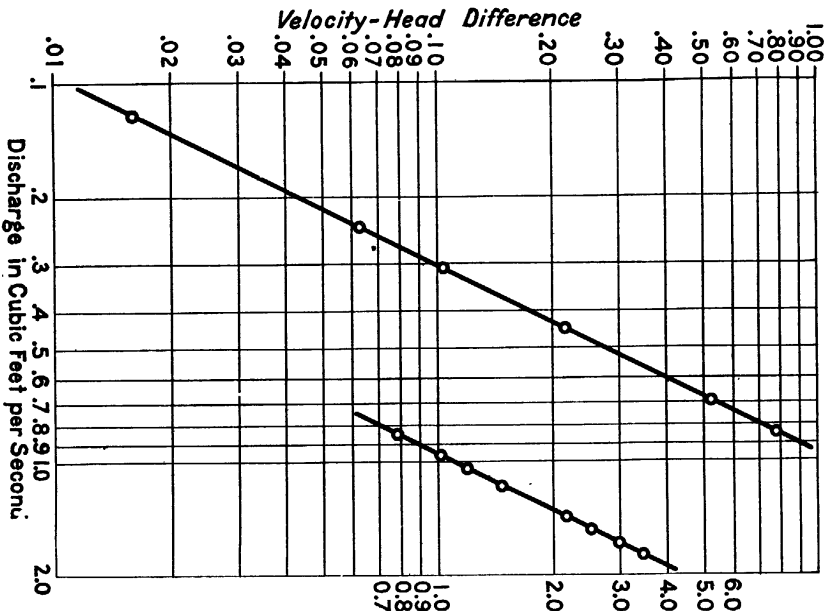


FIG. 4.—Velocity-head curve, pump No. 2, deep-well turbine pump, 5-stage, 14-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.1085 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.2670 square foot

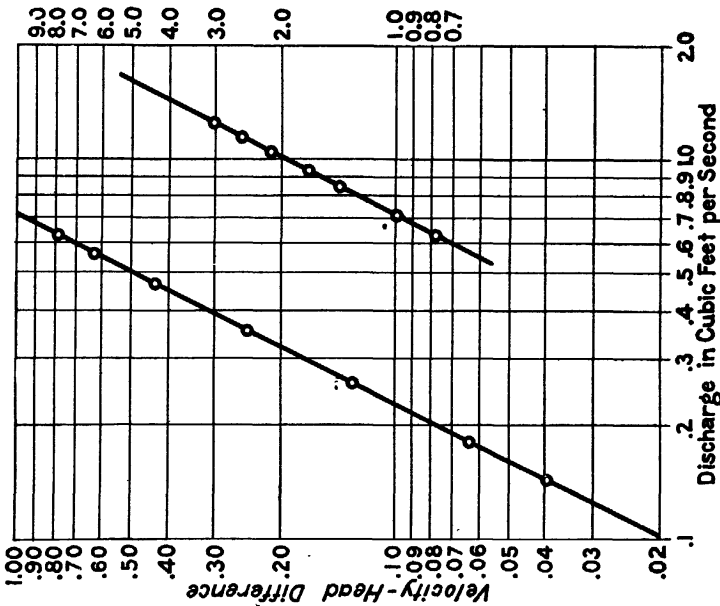


Fig. 5.—Velocity-head curve, pump No. 3, deep-well turbine pump, 7-stage, 12-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.0841 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.2670 square foot

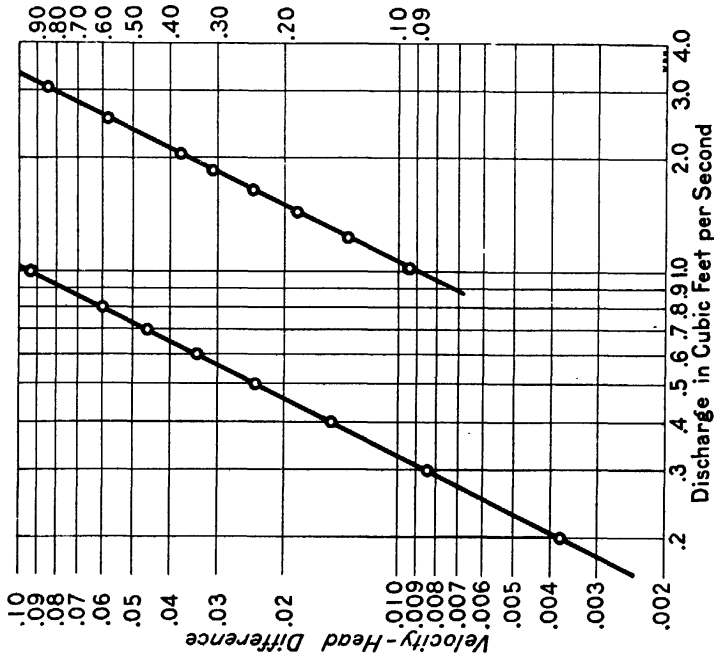


Fig. 6.—Velocity-head curve, pump No. 4, deep-well turbine pump, 2-stage, 24-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge pipe is attached, 0.3078 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.4700 square foot

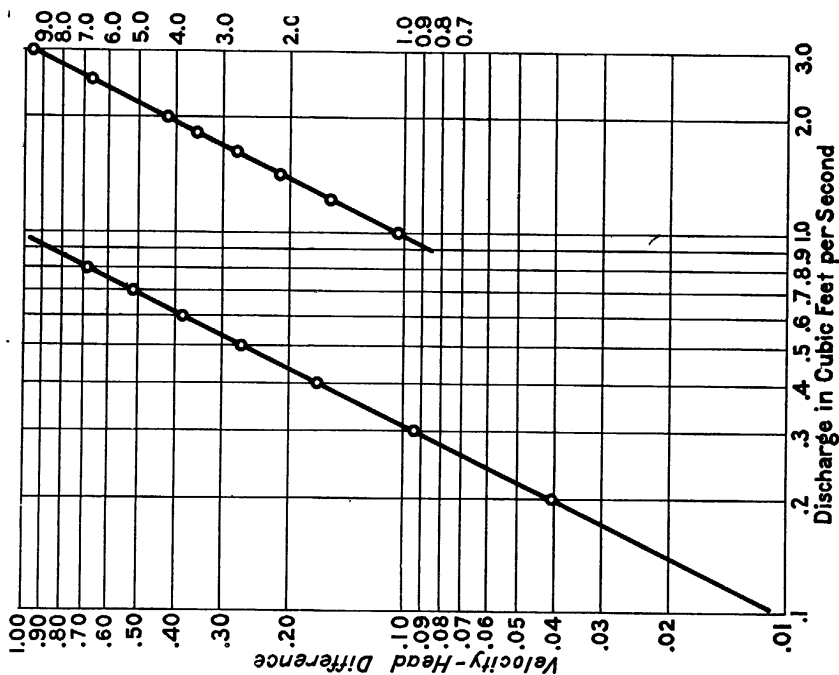


Fig. 7.—Velocity-head curve, pumps Nos. 5 and 6, deep-well turbine pump, 4-stage, 17-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge is attached, 0.108 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.249 square foot.

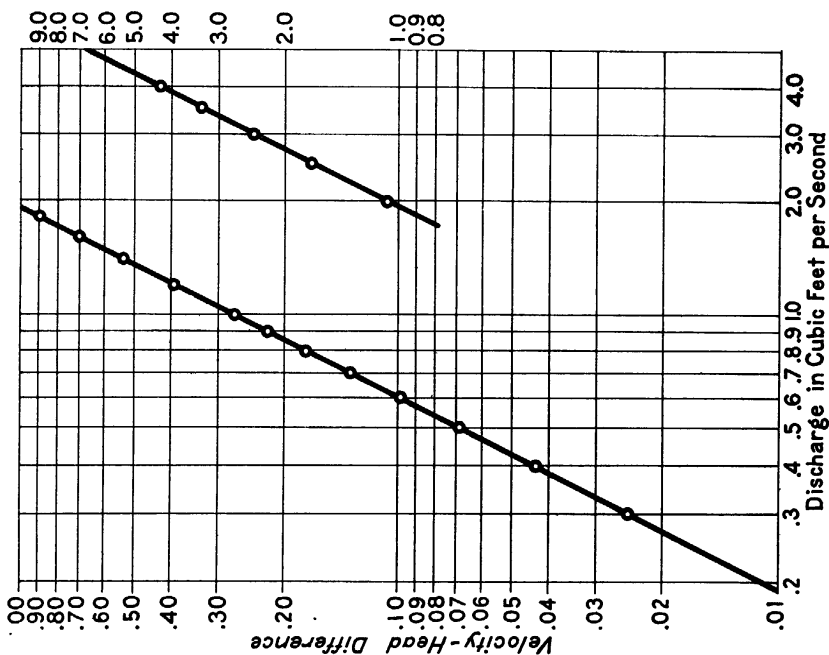


Fig. 8.—Velocity-head curve, pump No. 7, deep-well turbine pump, 4-stage, 17-inch diameter. Log curves show velocity-head difference between suction and discharge. Net area of suction where vacuum-gauge is attached, 0.197 square foot. Net area of discharge where pressure-gauge pipe is attached, 0.349 square foot.

TABLE I.—Layne & Bowler 3-stage, 24-inch pump, No. 1, tested October 24 to December 4, 1914

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
R. p. m.	R. p. m.		Ft.	Ft.	Ft.	Ft.	Ft.		G p.m.		P. ct.
686	688	1	58.05	0.00	15.50	-0.00	73.55	7.62	0	0.00	0.0
	685	2	59.80	1.62	15.50	-0.05	76.87	10.82	187	3.62	33.5
	686	3	53.40	4.15	15.50	-0.24	72.81	14.34	423	7.76	54.1
	685	4	45.34	5.52	15.50	-0.37	65.99	15.21	532	8.86	58.2
	684	5	38.20	6.71	15.50	-0.49	59.92	15.52	604	9.13	58.8
	685	6	32.11	7.75	15.50	-0.60	54.76	15.78	672	9.29	58.9
	686	7	26.53	8.57	15.50	-0.69	49.91	15.92	722	9.09	57.0
	687	8	20.45	9.48	15.50	-0.78	44.65	16.12	772	8.70	53.9
	686	9	13.45	10.36	15.50	-0.91	38.40	16.24	825	8.00	49.3
	686	10	6.12	11.17	15.50	-1.01	31.78	16.36	869	6.97	42.5
762	772	1	75.20	.39	15.50	-0.00	91.09	9.10	0	.00	.0
	765	2	78.70	2.03	15.50	-0.04	96.19	12.86	178	4.32	33.5
	766	3	73.28	4.48	15.50	-0.24	93.02	17.24	421	9.88	57.2
	759	4	68.90	4.46	15.50	-0.29	88.57	18.16	468	10.45	57.5
	760	5	59.95	6.21	15.50	-0.44	81.22	19.76	582	11.93	60.3
	760	6	49.75	7.91	15.50	-0.63	72.53	20.72	690	12.63	60.9
	761	7	43.80	8.92	15.50	-0.72	67.50	20.38	741	12.62	61.8
	761	8	35.80	10.00	15.50	-0.88	60.42	21.15	811	12.36	58.3
	760	9	28.60	11.01	15.50	-1.01	54.10	21.58	870	11.88	55.0
	758	10	21.74	11.78	15.50	-1.09	47.93	21.69	908	10.98	50.6
826	762	11	16.71	12.37	15.50	-1.16	43.42	21.72	937	10.26	47.2
	760	12	11.19	12.94	15.50	-1.25	38.38	20.92	970	9.39	44.8
	761	13	6.80	13.47	15.50	-1.30	34.47	21.14	992	8.63	40.8
	837	1	92.85	.37	15.50	-0.00	108.72	8.08	0	.00	.0
	834	2	96.80	2.51	15.50	-0.09	114.72	17.88	266	7.70	43.0
	828	3	88.45	5.00	15.50	-0.30	108.65	22.81	480	13.16	57.6
	826	4	78.90	6.62	15.50	-0.46	100.56	23.70	593	15.02	63.4
	823	5	73.05	7.56	15.50	-0.56	95.55	24.02	650	15.67	65.2
	822	6	62.40	9.02	15.50	-0.74	86.18	24.69	745	16.20	65.5
	824	7	57.20	9.81	15.50	-0.82	81.69	25.24	786	16.20	64.1
898	824	8	50.20	10.79	15.50	-0.95	75.54	25.20	849	16.29	64.6
	824	9	42.90	11.75	15.50	-1.07	69.08	25.60	899	15.67	61.1
	821	10	36.50	12.57	15.50	-1.18	63.39	26.15	943	15.08	57.6
	822	11	28.90	13.41	15.50	-1.28	56.53	26.27	983	14.02	53.3
	823	12	22.40	14.04	15.50	-1.35	50.59	26.01	1,010	12.89	49.5
	827	13	16.27	14.86	15.50	-1.47	45.16	26.16	1,055	12.02	45.9
	826	14	11.59	14.88	15.50	-1.53	40.44	26.73	1,075	10.97	41.0
	828	15	7.01	15.46	15.50	-1.61	36.36	26.43	1,102	10.11	38.1
	912	1	112.65	.45	15.50	-0.00	128.60	9.48	0	.00	.0
	903	2	115.51	3.16	15.50	-0.14	134.03	22.26	318	10.75	48.3
999	898	3	109.50	4.64	15.50	-0.29	129.35	26.95	468	15.26	56.6
	896	4	101.85	6.11	15.50	-0.44	123.02	28.50	577	17.91	62.8
	896	5	94.90	7.24	15.50	-0.56	117.08	29.79	646	19.08	64.0
	897	6	85.80	8.49	15.50	-0.70	109.19	30.64	727	20.03	65.3
	895	7	80.10	9.43	15.50	-0.79	104.24	31.14	775	20.39	65.4
	895	8	72.00	10.57	15.50	-0.95	97.12	32.03	846	20.73	64.7
	896	9	66.97	11.33	15.50	-1.06	92.74	32.08	891	20.85	64.9
	895	10	57.80	12.49	15.50	-1.19	84.60	32.59	945	20.17	61.8
	893	11	50.50	13.21	15.50	-1.33	77.88	33.36	998	19.61	58.7
	896	12	45.16	13.92	15.50	-1.40	73.18	33.28	1,028	18.98	57.0
1,011	899	13	38.53	14.86	15.50	-1.51	67.38	33.84	1,068	18.16	53.6
	897	14	31.40	15.53	15.50	-1.61	60.82	33.60	1,106	16.97	50.5
	897	15	26.34	16.12	15.50	-1.69	56.27	34.32	1,130	16.04	46.7
	898	16	18.97	16.94	15.50	-1.79	49.62	34.32	1,165	14.59	42.5
	895	17	13.34	17.46	15.50	-1.87	44.43	33.98	1,190	13.34	39.2
	897	18	7.56	17.90	15.50	-1.93	39.03	33.73	1,210	11.91	35.3
	1,026	1	147.50	.00	17.54	-0.00	165.04	18.12	0	.00	.0
	1,026	2	148.70	.73	17.54	-0.01	166.96	21.58	94	3.96	18.3
	1,020	3	151.18	1.55	17.54	-0.05	170.22	25.51	192	8.24	32.3
	1,025	4	152.75	2.54	17.54	-0.11	172.72	29.09	282	12.29	42.3
1,011	1,018	5	148.20	3.50	17.54	-0.18	169.06	32.29	365	15.57	48.2
	1,009	6	142.85	5.14	17.54	-0.32	165.21	36.07	493	20.55	56.9
	1,012	7	135.83	7.04	17.54	-0.51	159.90	40.28	624	25.18	62.5
	1,009	8	125.00	8.48	17.54	-0.67	150.35	42.04	710	26.94	64.1
	1,011	9	119.33	9.49	15.50	-0.78	143.54	42.97	766	27.75	64.5
	1,009	10	115.37	9.81	15.50	-0.87	139.81	45.02	807	28.47	63.2
	1,009	11	106.68	11.10	15.50	-1.00	132.28	45.74	866	28.91	63.2
	1,009	12	100.12	11.92	15.50	-1.12	126.42	46.67	921	29.38	62.9
	1,012	13	91.44	13.39	15.50	-1.29	119.04	48.10	986	29.62	61.6
	1,010	14	80.80	14.69	15.50	-1.46	109.53	48.22	1,049	28.99	60.0
1,011	1,009	15	70.30	15.93	15.50	-1.61	100.12	48.62	1,100	27.79	57.1
	1,008	16	61.93	16.69	15.50	-1.76	92.36	48.80	1,150	26.80	54.9
	1,008	17	57.06	17.37	15.50	-1.82	88.11	48.98	1,169	25.98	53.0
	1,006	18	49.16	18.16	15.50	-1.95	80.87	48.34	1,202	24.54	50.7
	1,004	19	37.86	19.24	15.50	-2.05	70.55	48.22	1,246	22.18	45.9
	1,003	20	31.41	19.78	15.50	-2.13	64.56	47.46	1,274	20.75	43.7
	1,003	21	21.47	20.51	15.50	-2.18	55.30	47.39	1,287	17.96	37.9
	999	22	8.48	21.19	15.50	-2.31	42.86	46.82	1,321	14.29	30.5

* Static head of mercury column=17.54 feet. Static head of pressure gauge=15.50 feet.

TABLE II.—Layne & Bowler 5-stage, 14-inch pump, No. 2, tested March 22 to April 15, 1915

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G.p.m.</i>		<i>P. ct.</i>
880	890	1	83.00	1.13	13.58	-0.00	97.71	6.46	0	0.00	0.0
	887	2	88.90	1.47	13.58	-.02	103.93	7.58	55	1.44	19.0
	886	3	86.67	2.49	13.58	-.14	102.60	9.88	162	4.19	42.4
	882	4	81.85	3.28	13.58	-.30	98.41	11.76	235	5.83	49.6
	882	5	75.30	4.24	13.58	-.50	92.62	12.45	305	7.13	57.3
	878	6	71.88	4.50	13.58	-.55	89.41	13.41	319	7.19	53.6
	878	7	62.20	5.37	13.58	-.79	80.36	14.11	382	7.74	54.8
	878	8	55.10	6.04	13.58	-.95	73.77	13.82	421	7.83	56.7
	878	9	47.80	6.55	13.58	-1.12	66.81	14.31	456	7.68	53.7
	879	10	44.25	6.86	13.58	-1.19	63.50	13.76	472	7.56	54.9
	878	11	37.60	7.40	13.58	-1.33	57.25	14.27	498	7.19	50.3
	880	12	31.50	7.80	13.58	-1.47	51.41	14.63	520	6.74	46.1
	881	13	26.00	8.13	13.58	-1.57	46.14	14.04	538	6.26	44.6
	876	14	19.40	8.64	13.58	-1.69	39.93	14.06	559	5.63	40.0
	876	15	13.61	8.89	13.58	-1.80	34.28	13.83	574	4.96	35.9
	873	16	6.78	9.27	13.58	-1.90	27.73	13.87	595	4.16	30.0
	988	1	106.50	1.41	13.58	-.00	121.49	6.39	0	.00	.0
978	979	2	111.60	2.15	13.58	-.06	127.27	11.74	108	3.47	29.6
	974	3	108.04	3.17	13.58	-.22	124.57	14.02	202	6.35	45.2
	981	4	99.69	4.58	13.58	-.52	117.33	15.66	312	9.25	59.1
	977	5	89.85	5.54	13.58	-.78	108.19	17.28	379	10.35	59.8
	973	6	77.69	5.93	13.58	-.96	96.24	17.63	422	10.25	58.1
	976	7	68.70	6.84	13.58	-1.19	87.93	17.78	468	10.38	58.3
	976	8	62.30	7.40	13.58	-1.35	81.93	16.88	500	10.34	61.1
	979	9	56.20	7.91	13.58	-1.54	76.15	17.07	531	10.20	59.7
	976	10	48.90	8.48	13.58	-1.68	69.28	17.14	556	9.72	56.6
	972	11	43.25	8.87	13.58	-1.80	63.90	18.64	574	9.26	49.6
	974	12	37.45	9.38	13.58	-1.91	58.50	18.67	595	8.78	47.0
	976	13	31.90	9.77	13.58	-2.05	53.20	16.62	614	8.24	49.5
	982	14	26.00	10.17	13.58	-2.16	47.59	18.19	630	7.56	41.6
	980	15	18.64	10.65	13.58	-2.32	40.55	17.56	654	6.69	38.0
	982	16	12.94	10.96	13.58	-2.40	35.08	17.56	665	5.88	33.5
	980	17	6.55	11.19	13.58	-2.51	28.81	18.07	679	4.93	27.3
1,066	1,078	1	128.30	1.47	13.58	-.00	143.35	8.60	0	.00	.0
	1,075	2	137.85	2.20	13.58	-.05	153.58	12.78	97	3.75	29.3
	1,072	3	136.00	2.26	13.58	-.14	151.70	12.96	164	6.27	48.4
	1,068	4	128.20	3.73	13.58	-.45	145.06	17.15	290	10.62	61.8
	1,064	5	120.80	4.53	13.58	-.67	138.24	18.16	350	12.21	67.2
	1,064	6	112.15	5.45	13.58	-.90	130.28	20.61	406	13.35	64.7
	1,064	7	103.40	6.16	13.58	-1.06	122.08	20.74	445	13.71	66.0
	1,062	8	95.70	6.81	13.58	-1.24	115.12	21.19	477	13.85	65.3
	1,066	9	87.10	7.52	13.58	-1.39	106.81	22.62	508	13.69	60.4
	1,062	10	78.85	8.14	13.58	-1.62	98.95	23.25	544	13.58	58.4
	1,064	11	68.35	8.82	13.58	-1.82	88.93	23.49	579	12.99	55.3
	1,065	12	60.20	9.44	13.58	-2.02	81.20	23.32	609	12.48	53.4
	1,058	13	50.70	9.77	13.58	-2.11	71.94	23.20	622	11.29	48.6
	1,062	14	42.80	10.28	13.58	-2.27	64.39	23.14	649	10.55	45.5
	1,064	15	32.90	10.96	13.58	-2.46	54.98	23.08	672	9.32	40.3
	1,065	16	27.20	11.30	13.58	-2.62	49.46	22.96	693	8.65	37.6
	1,064	17	20.39	11.81	13.58	-2.72	43.06	22.51	706	7.67	34.0
1,161	1,068	18	7.07	12.45	13.58	-2.98	30.12	22.56	740	5.62	24.9
	1,174	1	161.60	1.30	16.58	-.00	179.48	11.40	0	.00	.0
	1,171	2	164.40	1.92	16.58	-.03	182.87	12.61	79	3.65	28.9
	1,164	3	156.50	3.64	16.58	-.34	176.38	21.48	250	11.11	51.7
	1,162	4	151.00	4.75	16.58	-.58	171.75	23.35	326	14.13	60.5
	1,164	5	142.80	5.33	16.58	-.81	163.90	26.07	387	16.00	61.3
	1,162	6	134.20	6.16	16.58	-1.08	155.91	26.68	434	17.08	63.9
	1,160	7	126.96	6.86	13.58	-1.21	146.19	26.97	473	17.45	64.6
	1,162	8	119.45	7.48	13.58	-1.37	139.14	27.55	505	17.73	64.3
	1,159	9	111.55	8.14	13.58	-1.53	131.74	28.29	533	17.72	62.7
	1,160	10	103.40	8.81	13.58	-1.75	124.04	28.68	565	17.68	61.6
	1,157	11	94.00	9.47	13.58	-1.93	115.12	28.73	596	17.32	60.2
	1,160	12	88.05	10.03	13.58	-2.12	109.54	29.07	624	17.25	59.3
	1,161	13	81.60	10.45	13.58	-2.22	103.41	28.98	641	16.72	57.6
	1,161	14	74.50	10.85	13.58	-2.40	96.63	29.06	665	16.20	55.7
	1,164	15	66.91	11.27	13.58	-2.51	89.25	27.86	682	15.36	55.0
	1,160	16	58.89	11.76	13.58	-2.67	81.56	28.83	700	14.41	49.9
	1,160	17	53.90	12.03	13.58	-2.75	76.76	28.02	709	13.73	49.0
	1,158	18	47.15	12.31	13.58	-2.83	70.21	28.00	722	12.79	45.6
	1,156	19	39.80	12.65	13.58	-2.91	63.12	28.56	734	11.69	40.9
	1,158	20	33.34	13.11	13.58	-3.10	56.93	28.80	754	10.82	37.6
	1,158	21	27.10	13.44	13.58	-3.16	50.96	28.68	762	9.80	34.1
	1,158	22	28.80	12.89	13.58	-3.11	52.16	29.08	754	9.92	34.1
	1,153	23	21.90	13.32	13.58	-3.21	45.59	28.93	768	8.83	30.5
	1,158	24	15.50	13.72	13.58	-3.28	39.62	29.03	776	7.74	26.6

• Static head for pressure gauge=16.58. Static head for mercury column=13.58.

TABLE III.—Layne & Bowler 7-stage, 12-inch pump, No. 3, tested January 27 to February 1, 1915

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horse-power	Dis-charge	Water horse-power	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G. p. m.</i>		<i>Per ct.</i>
987	990	1	87.00	0.90	14.15	-0.00	102.05	4.96	0	0.00	0.0
	990	2	90.20	1.53	14.15	-0.07	105.81	7.53	85	2.26	30.0
	990	3	89.49	1.41	14.15	-0.09	104.96	6.35	94	2.49	39.2
	984	4	80.80	2.06	14.15	-0.24	96.77	7.73	157	3.83	49.5
	986	5	73.70	2.66	14.15	-0.41	90.10	7.71	205	4.66	60.4
	989	6	65.50	3.33	14.15	-0.63	82.35	8.48	253	5.25	61.9
	988	7	59.95	3.73	14.15	-0.77	77.06	8.07	279	5.42	67.2
	985	8	53.35	4.18	14.15	-0.93	70.75	8.50	306	5.46	64.2
	988	9	46.60	4.63	14.15	-1.10	64.28	7.95	334	5.41	68.1
	988	10	39.60	5.14	14.15	-1.25	57.54	7.91	359	5.21	65.9
	988	11	33.65	5.48	14.15	-1.42	51.86	7.75	381	4.98	64.3
	986	12	25.55	5.93	14.15	-1.60	44.03	7.52	404	4.48	59.6
	982	13	5.42	7.06	14.15	-2.07	24.56	6.96	461	2.85	40.9
	1,028	1	98.30	0.51	14.15	-0.00	112.96	6.76	0	0.00	0.0
1,030	1,030	2	101.10	1.13	14.15	-0.06	116.32	9.40	81	2.37	25.2
	1,032	3	93.60	1.64	14.15	-0.18	109.21	10.16	136	3.75	36.8
	1,031	4	83.10	2.49	14.15	-0.43	99.31	11.70	209	5.23	44.7
	1,030	5	60.65	4.17	14.15	-0.97	78.00	10.46	313	6.16	58.8
	1,029	6	45.35	5.13	14.15	-1.34	63.29	10.04	370	5.91	58.8
	1,031	7	37.80	5.65	14.15	-1.55	55.55	9.82	397	5.56	56.6
	1,031	8	31.41	5.87	14.15	-1.69	49.74	10.52	416	5.22	49.5
	1,030	9	23.56	6.55	14.15	-1.86	42.40	9.47	437	4.67	49.3
	1,029	10	14.92	7.01	14.15	-2.10	33.98	9.49	464	3.97	41.8
	1,032	11	5.71	7.57	14.15	-2.35	25.08	8.82	488	3.08	34.9
	1,078	1	108.17	0.85	14.15	-0.00	123.17	7.35	0	.00	.0
	1,078	2	112.60	1.24	14.15	-0.04	127.95	8.46	63	2.03	24.0
	1,070	3	100.80	2.20	14.15	-0.26	116.89	11.27	162	4.77	42.3
	1,073	4	90.80	2.77	14.15	-0.47	107.25	12.94	220	5.95	46.0
1,073	1,066	5	81.60	3.50	14.15	-0.70	98.55	13.16	267	6.64	50.4
	1,068	6	70.40	4.21	14.15	-0.98	87.78	13.36	315	6.97	52.2
	1,071	7	63.20	4.77	14.15	-1.16	80.96	13.08	343	7.00	53.5
	1,073	8	56.10	5.25	14.15	-1.34	74.16	13.30	370	6.92	52.0
	1,072	9	49.20	5.71	14.15	-1.52	67.54	13.13	395	6.73	51.2
	1,073	10	42.40	6.10	14.15	-1.69	60.96	12.43	416	6.40	51.4
	1,072	11	34.85	6.55	14.15	-1.85	53.70	10.72	435	5.89	54.9
	1,073	12	26.34	7.06	14.15	-2.07	45.48	10.26	461	5.29	51.5
	1,076	13	13.30	7.85	14.15	-2.40	32.90	9.06	490	4.06	44.8
	1,073	14	5.76	8.28	14.15	-2.67	25.52	8.81	518	3.33	37.8
	1,171	1	130.25	0.56	14.15	-0.00	144.96	7.64	0	.00	.0
	1,168	2	131.85	1.47	14.15	-0.13	147.34	12.10	114	4.23	35.0
	1,170	3	123.70	2.12	14.15	-0.29	139.68	11.89	174	6.13	51.6
	1,170	4	115.65	2.66	14.15	-0.46	132.00	12.43	218	7.26	58.3
1,168	1,164	5	106.65	3.33	14.15	-0.67	123.46	13.42	261	8.13	60.5
	1,166	6	97.05	3.95	14.15	-0.90	114.25	15.82	302	8.70	54.9
	1,166	7	85.15	4.69	14.15	-1.17	102.82	15.74	345	8.95	56.8
	1,162	8	77.60	5.20	14.15	-1.35	95.60	16.16	372	8.97	55.5
	1,164	9	70.23	5.65	14.15	-1.50	88.53	16.43	391	8.73	53.1
	1,169	10	64.40	6.10	14.15	-1.69	82.96	16.02	416	8.70	54.3
	1,167	11	55.70	6.55	14.15	-1.90	74.50	15.68	440	8.27	52.7
	1,165	12	48.80	6.95	14.15	-2.08	67.82	15.74	461	7.89	50.1
	1,172	13	47.50	6.95	14.15	-2.16	66.44	16.22	468	7.84	48.3
	1,172	14	40.70	7.40	14.15	-2.35	59.90	15.66	490	7.40	47.2
	1,172	15	35.20	7.74	14.15	-2.49	54.60	15.32	505	6.95	45.4
	1,168	16	29.94	8.02	14.15	-2.65	49.46	15.40	515	6.42	41.7
	1,168	17	22.60	8.48	14.15	-2.82	42.41	14.79	533	5.70	38.5
	1,171	18	16.05	8.87	14.15	-2.97	36.10	14.76	551	5.02	33.9
	1,172	19	7.23	9.38	14.15	-3.16	27.60	14.12	569	3.96	28.0

TABLE IV.—American 2-stage, 24-inch pump, No. 4, tested January 29, March 9, March 11, 1916

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G.p.m.</i>		<i>P. ct.</i>
890	898	1	93.37	0.00	14.98	—0.00	108.35	12.92	0	0.00	0.0
	893	2	87.58	.00	14.98	— .01	102.55	17.82	142	3.67	20.5
	892	3	78.14	1.07	14.98	— .04	94.15	22.04	313	7.43	33.7
	889	4	70.18	3.05	14.98	— .11	88.10	24.08	492	10.94	45.4
	888	5	60.46	5.22	14.98	— .20	80.46	28.11	657	13.36	47.5
	885	6	51.64	7.48	14.98	— .34	73.76	29.95	807	15.06	50.3
	885	7	43.96	9.74	14.98	— .40	68.28	30.19	936	16.13	53.5
	888	8	35.14	12.23	14.98	— .53	61.82	30.39	1,084	16.92	55.6
	889	9	27.12	14.69	14.98	— .66	56.13	30.87	1,206	17.09	55.3
	889	10	14.12	17.09	14.98	— .82	45.37	30.70	1,332	15.26	50.6
	889	11	12.03	19.29	14.98	— .92	45.38	27.00	1,408	16.13	59.6
	892	12	73.96	2.32	14.98	— .07	91.19	21.19	391	9.00	42.5
	888	13	63.22	4.80	14.98	— .16	82.84	26.47	594	12.42	46.8
	936	1	105.09	.00	14.98	— .00	120.07	17.24	0	.00	.0
928	934	2	96.95	.14	14.98	— .02	112.05	20.93	181	5.11	24.4
	932	3	87.58	1.31	14.98	— .06	103.81	24.49	358	9.38	38.3
	929	4	78.80	2.77	14.98	— .11	96.44	26.38	487	11.85	44.9
	929	5	69.05	4.89	14.98	— .20	88.72	29.44	654	14.65	49.7
	927	6	60.22	8.13	14.98	— .29	83.04	32.18	792	16.59	51.6
	926	7	51.70	9.46	14.98	— .40	75.74	33.96	933	17.84	52.5
	926	8	44.24	11.64	14.98	— .51	70.35	34.60	1,058	18.79	54.2
	924	9	35.48	14.18	14.98	— .64	64.00	35.20	1,183	19.11	54.2
	923	10	27.01	16.56	14.98	— .77	57.78	35.67	1,300	18.96	53.1
	926	11	11.75	20.28	14.98	— .98	46.03	33.57	1,465	17.03	50.7
	995	1	120.97	.00	14.98	— .00	135.95	19.11	0	.00	.0
	993	2	114.46	.00	14.98	— .01	129.43	23.66	131	4.30	18.2
	993	3	109.27	.48	14.98	— .03	124.70	26.07	241	7.57	29.0
	987	4	102.55	.99	14.98	— .04	118.48	28.97	305	9.11	31.4
987	990	5	99.89	2.15	14.98	— .08	116.94	31.58	403	11.88	37.6
	990	6	91.98	3.05	14.98	— .11	109.90	33.23	487	13.51	40.6
	987	7	84.09	5.08	14.98	— .19	103.96	36.50	640	16.80	46.0
	984	8	73.96	7.03	14.98	— .28	95.69	38.46	778	18.79	48.8
	984	9	65.94	9.35	14.98	— .37	89.90	40.43	899	20.40	50.4
	982	10	62.37	10.38	14.98	— .43	87.30	40.84	967	21.31	52.1
	984	11	53.56	12.60	14.98	— .54	80.60	42.05	1,097	22.32	53.0
	982	12	45.19	14.95	14.98	— .66	74.46	42.44	1,199	22.54	53.1
	981	13	36.89	17.40	14.98	— .80	68.47	42.10	1,321	22.83	54.2
	985	14	13.05	20.31	14.98	—1.09	47.25	39.50	1,468	17.55	44.4
	1,090	1	143.77	.00	18.56	— .00	162.33	22.82	0	.00	.0
	1,084	2	136.50	.00	14.98	— .01	151.47	29.20	150	5.73	19.6
	1,080	3	129.66	.45	14.98	— .03	145.06	33.73	267	9.76	28.9
	1,075	4	118.65	2.09	14.98	— .08	135.64	39.04	416	14.24	36.4
1,071	1,073	5	111.19	3.73	14.98	— .14	129.76	42.78	551	18.05	42.1
	1,068	6	101.58	5.82	14.98	— .22	122.16	45.70	699	21.56	47.1
	1,069	7	93.00	7.63	14.98	— .30	115.31	48.27	818	23.81	49.3
	1,068	8	84.52	9.72	14.98	— .40	108.82	50.13	933	25.63	51.1
	1,065	9	83.73	9.38	14.98	— .40	107.69	50.68	940	25.56	50.3
	1,063	10	76.05	11.45	14.98	— .50	101.98	51.42	1,046	26.93	52.3
	1,064	11	66.44	13.90	14.98	— .64	94.68	52.75	1,176	28.11	53.2
	1,062	12	58.37	15.93	14.98	— .77	88.54	52.67	1,280	28.61	54.2
	1,061	13	50.51	17.79	14.98	— .90	82.38	52.51	1,401	29.17	55.4
	1,066	14	41.24	19.61	14.98	— .99	74.87	51.88	1,466	27.1	53.3

TABLE V.—American 4-stage, 17-inch pump, No. 5, tested March 20 and March 28, 1916

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G.p.m.</i>		<i>P. ct.</i>
857	865	1	105.66	1.46	11.59	—0.00	118.71	2.60	0	0.00	0.0
	860	2	90.96	2.71	11.59	— .10	105.16	11.30	137	3.63	32.1
	856	3	81.65	3.73	11.59	— .27	96.70	14.02	225	5.48	39.2
	854	4	74.23	4.78	11.59	— .50	90.10	16.74	309	7.02	41.9
	853	5	64.07	6.98	11.59	—1.01	81.63	18.57	435	8.95	48.2
	853	6	56.96	8.50	11.59	—1.36	75.69	18.04	505	9.65	53.4
	854	7	47.06	10.48	11.59	—1.86	67.27	18.64	590	10.02	53.8
	855	8	38.25	12.29	11.59	—2.30	59.83	17.65	657	9.91	56.2
	856	9	29.15	14.46	11.59	—2.86	52.34	15.97	732	9.66	60.4
	857	10	20.57	16.58	11.59	—3.34	45.40	16.39	792	9.06	55.3
	858	11	8.23	20.28	11.59	—4.32	35.78	14.91	900	8.12	54.5
934	945	1	123.85	1.64	11.59	— .00	137.08	4.07	0	.00	.0
	940	2	111.47	2.85	11.59	— .12	125.79	9.93	149	4.72	47.5
	934	3	101.08	3.98	11.59	— .28	116.37	15.51	231	6.77	43.6
	933	4	92.20	5.11	11.59	— .55	108.35	20.98	325	8.87	42.3
	932	5	82.89	6.92	11.59	—1.10	100.30	23.01	455	11.50	49.9
	930	6	73.18	8.95	11.59	—1.46	92.26	24.17	523	12.16	50.3
	931	7	62.99	10.85	11.59	—1.90	83.53	24.05	596	12.55	52.2
	933	8	47.57	14.12	11.59	—2.73	70.55	23.66	716	12.73	53.8
	933	9	25.14	19.52	11.59	—4.17	52.08	22.43	884	11.62	51.7
	935	10	8.14	24.66	11.59	—5.47	38.92	16.15	1,012	9.93	61.5
	1,090	1	183.08	2.04	14.57	— .00	199.69	16.55	0	.00	.0
1,079	1,085	2	159.44	2.68	14.57	— .06	176.63	18.97	108	4.81	25.4
	1,081	3	145.15	3.39	14.57	— .21	162.90	24.04	201	8.25	34.3
	1,081	4	138.14	4.55	11.59	— .43	153.85	27.98	286	11.09	39.6
	1,078	5	124.20	6.35	11.59	— .91	141.23	33.57	411	14.63	43.6
	1,076	6	111.70	8.76	11.59	—1.59	130.46	36.14	536	17.63	48.8
	1,074	7	97.63	11.48	11.59	—2.21	118.49	37.31	644	19.26	51.5
	1,075	8	83.05	14.24	11.59	—2.95	105.93	37.78	744	19.89	52.6
	1,075	9	68.93	17.12	11.59	—3.67	93.97	37.31	829	19.66	52.6
	1,076	10	56.16	20.02	11.59	—4.35	83.42	36.11	903	19.01	52.6
	1,078	11	43.56	23.11	11.59	—5.12	73.14	34.50	980	18.09	52.3
	1,076	12	30.17	26.72	11.59	—6.13	62.35	33.22	1,071	16.85	50.7
1,169	1,077	13	8.70	29.04	11.59	—6.55	42.78	32.33	1,108	11.95	36.9
	1,186	1	220.83	2.43	14.57	— .00	237.83	14.28	0	.00	.0
	1,178	2	197.67	2.88	14.57	— .04	215.08	24.16	94	5.09	21.1
	1,177	3	184.32	3.81	14.57	— .14	202.56	28.26	165	8.42	29.8
	1,176	4	171.76	4.24	14.57	— .30	190.27	32.42	241	11.57	35.7
	1,171	5	155.24	5.78	14.57	— .72	174.87	38.62	366	16.15	41.8
	1,166	6	135.95	8.74	14.57	—1.52	157.74	43.57	534	21.26	48.7
	1,164	7	119.90	12.17	11.59	—2.38	141.28	45.70	668	23.81	52.0
	1,158	8	101.46	15.48	11.59	—3.20	125.33	46.08	775	24.51	53.1
	1,158	9	84.01	19.07	11.59	—4.12	110.55	45.67	878	24.49	53.6
	1,163	10	65.31	23.36	11.59	—5.22	95.04	44.63	989	23.72	53.2
	1,165	11	46.08	27.68	11.59	—6.36	78.99	43.89	1,092	21.77	49.5

In order to facilitate the plotting of the performance curves, characteristic curves similar to those shown in Figure 9 were plotted for each average speed of pump. The efficiency, total head, speed, brake horsepower, and water horsepower were plotted as ordinates, and the corresponding discharges in gallons per minute were plotted as abscissas from data given in Tables 1 to 7, inclusive. From these characteristic curves, the performance curves were produced by plotting the capacity-head curves for the various speeds of each pump on the same sheet. Points of equal efficiency taken from the characteristic curves were connected so as to form iso-efficiency curves. In the same manner points of equal brake horsepower were taken from the characteristic curves and connected to form curves showing points of equal brake horsepower.

TABLE VI.—American 2-stage, 17-inch pump, No. 6

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G.p.m.</i>		<i>P. ct.</i>
768	768	1	36.16	0.56	11.62	—0.00	48.34	5.51	0	0.00	0.0
	766	2	32.09	1.24	11.62	—0.02	44.93	5.82	53	.60	10.3
	768	3	28.70	1.69	11.62	—0.07	41.94	6.13	112	1.18	19.2
	767	4	22.82	3.05	11.62	—0.29	37.20	7.16	236	2.21	30.9
	769	5	17.74	4.86	11.62	—0.66	33.56	7.86	350	2.96	37.7
	770	6	13.11	6.78	11.62	—1.05	30.46	8.31	446	3.43	41.3
	767	7	7.23	9.23	11.62	—1.76	26.81	8.22	572	3.87	47.1
	862	1	49.04	.23	11.62	—0.00	60.89	2.24	0	.00	.0
	856	2	42.94	1.18	11.62	—0.03	55.71	4.92	74	1.04	21.2
	854	3	39.66	1.69	11.62	—0.06	52.91	6.15	122	1.62	26.3
854	855	4	35.59	2.49	11.62	—0.21	49.49	6.40	198	2.47	38.6
	852	5	28.76	4.26	11.62	—0.58	44.06	8.46	333	3.70	43.7
	851	6	22.15	6.67	11.62	—1.15	39.29	10.60	464	4.60	43.4
	851	7	18.08	8.19	11.62	—1.52	36.37	10.09	533	4.89	48.5
	852	8	13.90	8.83	11.62	—1.92	32.43	10.26	601	4.93	48.0
	851	9	7.06	13.31	11.62	—2.66	29.33	10.44	711	5.26	50.3
	935	1	61.06	.28	11.62	—0.00	72.96	6.89	0	.00	.0
	938	2	55.48	1.13	11.62	—0.02	68.21	5.90	53	.91	15.4
	937	3	50.85	1.86	11.62	—0.07	64.26	8.40	115	1.86	22.1
	936	4	46.49	2.20	11.62	—0.18	60.13	8.32	186	2.82	33.9
934	932	5	39.55	3.84	11.62	—0.49	54.52	10.45	306	4.20	40.2
	932	6	31.98	6.41	11.62	—1.05	48.96	12.15	446	5.50	45.3
	930	7	27.57	8.05	11.62	—1.44	45.80	12.97	518	5.98	46.1
	931	8	20.45	10.73	11.62	—2.10	40.70	13.39	630	6.46	48.2
	932	9	14.06	13.56	11.62	—2.76	36.48	13.39	724	6.66	49.7
	932	10	7.32	16.72	11.62	—3.57	32.09	13.45	825	6.68	49.6
	1,004	1	69.21	.45	11.62	—0.00	81.28	5.90	0	.00	.0
	999	2	62.72	1.58	11.62	—0.02	75.90	6.65	71	1.36	20.5
	995	3	56.39	2.20	11.62	—0.12	70.09	9.27	153	2.70	29.1
	992	4	48.31	3.78	11.62	—0.41	63.30	11.77	280	4.47	38.0
995	990	5	39.60	6.78	11.62	—1.12	56.88	14.10	458	6.57	46.6
	992	6	30.74	10.40	11.62	—1.81	50.95	15.15	582	7.48	49.4
	993	7	22.83	13.73	11.62	—2.81	45.37	15.18	727	8.32	54.8
	993	8	10.17	19.16	11.62	—4.32	36.63	14.77	902	8.34	56.4
	1,093	1	84.18	.59	11.62	—0.00	96.39	6.71	0	.00	.0
	1,093	2	75.60	2.20	11.62	—0.04	89.38	10.80	87	1.96	18.1
	1,092	3	66.85	2.82	11.62	—0.18	81.11	11.35	186	3.80	33.5
	1,093	4	59.72	4.63	11.62	—0.48	75.49	11.68	300	5.70	48.8
	1,091	5	50.85	7.03	11.62	—1.04	68.46	17.22	444	7.66	44.5
	1,090	6	41.92	10.17	11.62	—1.82	61.87	19.11	584	9.14	47.8
1,086	1,080	8	44.41	9.04	11.62	—1.55	63.52	19.15	541	8.66	45.2
	1,080	9	37.23	11.64	11.62	—2.17	58.32	19.96	641	9.42	47.2
	1,082	10	36.61	12.20	11.62	—2.50	57.93	20.13	685	10.00	49.7
	1,082	11	29.27	15.03	11.62	—3.20	52.72	20.23	775	10.30	50.9
	1,082	12	22.31	18.08	11.62	—3.98	48.03	20.22	864	10.46	51.7
	1,082	13	16.84	20.28	11.62	—4.51	44.23	19.81	921	10.28	51.9
	1,082	14	8.19	24.35	11.62	—5.55	38.61	19.60	1,023	9.95	50.8
	1,191	1	103.55	.49	11.62	—0.00	115.66	2.40	0	.00	.0
	1,190	2	94.68	1.69	11.62	—0.02	107.97	8.82	62	1.69	19.2
	1,189	3	90.46	2.46	11.62	—0.08	104.46	11.73	124	3.26	27.8
1,184	1,189	4	84.18	2.82	11.62	—0.14	98.48	12.43	166	4.12	33.1
	1,188	5	79.16	3.28	11.62	—0.30	93.76	16.48	237	5.60	34.0
	1,183	6	72.07	4.89	11.62	—0.54	88.04	19.09	319	7.09	37.1
	1,186	7	68.93	5.31	11.62	—0.69	85.17	18.08	361	7.75	42.9
	1,182	8	64.64	6.33	11.62	—1.00	81.59	22.05	444	9.13	41.4
	1,182	9	59.78	7.91	11.62	—1.43	77.88	22.63	518	10.17	44.9
	1,180	10	54.80	9.83	11.62	—1.87	74.38	23.66	595	11.17	46.9
	1,180	11	44.24	13.56	11.62	—2.81	66.61	25.23	732	12.29	48.7
	1,176	12	37.52	16.10	11.62	—3.46	61.78	25.61	804	12.57	49.0
	1,180	13	30.51	19.89	11.62	—4.25	57.77	26.20	896	13.06	49.8
	1,180	14	22.31	24.01	11.62	—5.40	52.54	25.58	1,008	13.39	52.3
	1,178	15	13.00	27.37	11.62	—6.14	45.85	25.22	1,074	12.41	49.2

TABLE VII.—Byron-Jackson 4-stage, 17-inch pump, No. 7

Average pump speed	Speed of pump	No. of run	Pressure head	Suction head	Static head	Velocity head	Total head	Brake horsepower	Discharge	Water horsepower	Efficiency
<i>R. p. m.</i>	<i>R. p. m.</i>		<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>		<i>G.p.m.</i>		<i>P. ct.</i>
879	878	1	53.79	0.00	12.26	—0.00	66.05	9.09	0	0.00	0.0
	879	2	58.76	1.13	12.26	—0.02	72.13	9.70	109	1.98	20.4
	880	3	58.99	1.58	12.26	—0.07	72.76	12.13	230	4.22	34.8
	879	4	54.24	3.16	12.26	—0.25	69.41	14.46	437	7.64	52.8
	880	5	48.15	4.97	12.26	—0.42	64.96	15.00	561	9.18	61.2
	878	6	39.97	7.34	12.26	—0.66	58.91	11.63	701	10.42	89.6
	878	7	28.25	9.27	12.26	—0.86	48.92	11.46	804	9.91	86.5
	879	8	8.36	12.43	12.26	—1.23	31.82	12.91	964	7.73	59.9
	990	1	67.12	.23	12.26	—0.00	79.61	8.31	0	.00	.0
	995	2	76.61	1.13	12.26	—0.04	89.96	13.22	164	3.72	28.1
986	986	3	72.09	3.50	12.26	—0.29	87.56	18.97	464	10.24	53.9
	984	4	64.41	5.37	12.26	—0.46	81.58	20.30	588	12.09	59.6
	988	5	56.05	7.57	12.26	—0.67	75.21	20.05	707	13.42	66.8
	981	6	47.23	8.95	12.26	—0.84	67.58	20.05	798	13.64	67.8
	985	7	39.55	10.40	12.26	—1.02	61.19	20.05	873	13.48	67.2
	982	8	30.06	11.98	12.26	—1.20	53.10	19.83	949	12.70	64.0
	985	9	19.21	13.79	12.26	—1.46	43.80	19.42	1,045	11.55	59.4
	982	10	9.04	15.14	12.26	—1.59	34.85	19.20	1,094	9.61	50.1
	1,086	1	82.26	.00	12.26	—0.00	94.52	13.29	0	.00	.0
	1,083	2	92.89	.45	12.26	—0.01	105.59	14.35	93	2.47	17.2
1,073	1,075	3	93.11	1.13	12.26	—0.06	106.44	17.95	211	5.66	31.5
	1,068	4	90.85	2.71	12.26	—0.22	105.60	22.19	404	10.75	48.4
	1,070	5	82.04	5.42	12.26	—0.47	99.25	25.17	593	14.85	58.9
	1,074	6	74.35	7.57	12.26	—0.69	93.49	26.58	716	16.89	63.5
	1,071	7	63.28	9.61	12.26	—0.90	84.25	25.78	825	17.54	68.0
	1,069	8	50.62	11.64	12.26	—1.14	73.38	26.12	927	17.16	65.6
	1,069	9	32.32	14.46	12.26	—1.50	57.54	24.69	1,058	15.34	62.1
	1,067	10	11.75	18.08	12.26	—1.93	40.16	23.77	1,202	12.18	51.2
	1,162	1	112.44	.00	12.26	—0.00	124.70	16.46	0	.00	.0
	1,165	2	112.77	.23	12.26	—0.00	125.26	17.04	48	1.52	8.9
1,155	1,149	3	111.42	1.13	12.26	—0.05	124.76	20.98	199	6.27	29.9
	1,150	4	102.60	4.92	12.26	—0.43	119.35	29.34	564	16.98	57.8
	1,154	5	94.69	6.55	12.26	—0.60	112.90	30.89	671	19.12	61.7
	1,148	6	90.85	7.57	12.26	—0.70	109.98	31.22	727	20.18	64.5
	1,153	7	83.39	9.64	12.26	—0.86	103.83	31.77	805	21.09	66.3
	1,153	8	70.06	11.75	12.26	—1.17	92.90	32.89	939	22.01	66.8
	1,155	9	44.97	15.93	12.26	—1.71	71.45	31.80	1,126	20.30	63.7
	1,155	10	33.00	18.36	12.26	—1.98	61.64	31.84	1,219	18.96	59.6
	1,161	11	21.70	19.55	12.26	—2.24	51.27	30.55	1,280	16.55	54.2

DISCUSSION OF TYPICAL CHARACTERISTIC CURVES

A typical characteristic diagram, including a capacity-head curve, an efficiency curve, brake and water horsepower curve, and a speed curve, is shown as Figure 9, page 241, representing the various characteristics for pump No. 1 when run at an average speed of 898 revolutions per minute.

CAPACITY-HEAD CURVE

The capacity-head curve represents the relation between the total heads pumped against and the corresponding quantities of water in gallons per minute which the pump is capable of delivering when operated (in this instance) at an average speed of 898 revolutions per minute. The pump was started with zero discharge, i. e., the gate-valve control was closed and an initial total head of 128.6 feet was attained (see Table I). As the valve was slowly opened, the head gradually increased until a flow of 318 gallons per minute was reached with a total measured head of 134 feet. The head then dropped gradually, upon further opening of the control valve, until it came again to the measured total head of 129.3 feet, but with the discharge increased to 468 gallons per minute.

Thus it is seen that in this portion of the capacity-head curve there are two capacities of the pump for heads ranging from 128.6

to 134 feet for the average speed of 898 revolutions per minute. The stability of the operation of the pump between these limits is due to the balancing effect of the friction in the discharge pipe. As the head lowers, the capacity increases until a point is reached where the control-gate valve is wide open. At this point the discharge is maximum at 1,210 gallons per minute. The head would have been zero at this point of opening of the gate valve if the pump had been discharging into the air instead of raising the water high enough to pass through the measuring weir. Under such conditions, with zero head, no useful work would have been performed by the pump.

EFFICIENCY CURVE

With no flow from the pump at the start, no useful work is being done by the pump, and hence the efficiency or ratio of output to input of power is zero. The efficiency curve therefore starts at zero. As the gate is opened, the capacity becomes greater and the efficiency increases very rapidly until a point of maximum efficiency of 65.4 per cent is reached. At this point the capacity is 775 gallons per minute. When the maximum capacity is reached the head is zero, with no useful work being done by the pump, and the efficiency also is zero.

A point of interest in connection with this curve is that for a given efficiency there are two heads with their respective capacities. For example: With an efficiency of 60 per cent, the corresponding heads are 127 feet and 78 feet, with capacities of 510 and 985 gallons per minute, respectively. The data show a wide range of heads and capacities for efficiencies of 60 per cent or greater. By referring to Figure 9, the range of heads and capacities may be noted for various efficiencies of 40 per cent and over.

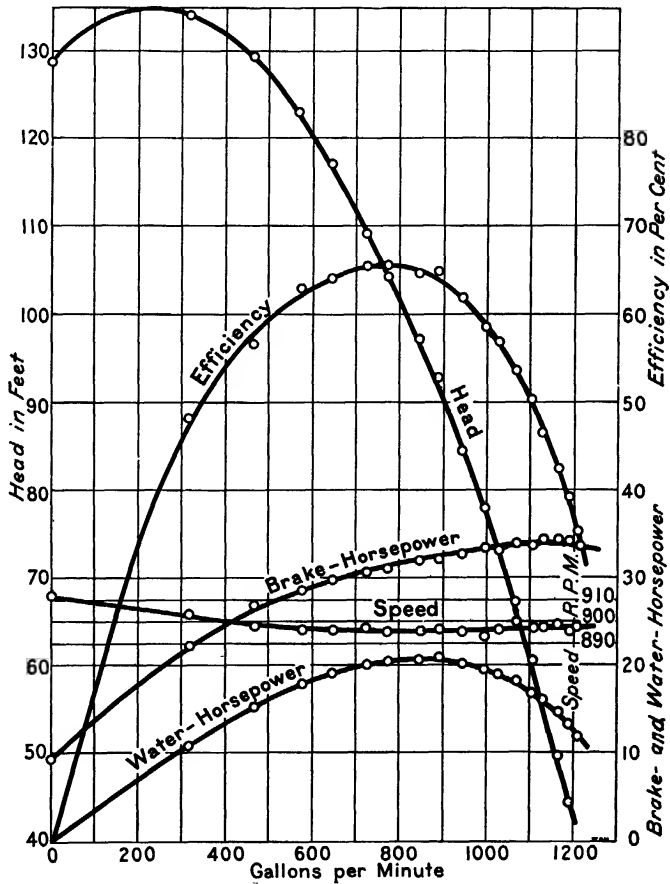


FIG. 9.—Characteristic curves, pump No. 1, 3-stage, 24-inch diameter, average speed 898 r. p. m.

BRAKE-HORSEPOWER CURVE

From this curve the size of prime mover to select can be determined, and the load which will be carried for any given set of con-

ditions ascertained. The brake-horsepower curve does not start at zero as do the capacity-head and efficiency curves, as indicated in Figure 9. For zero flow, the power consumed amounted to 9.48 horsepower, and the pump speed was 912 revolutions per minute, although the pump did no useful work. The curve reaches a maximum at 34.3 horsepower, with a total head of 56.3 feet and a flow of 1,130 gallons per minute. At the point of maximum capacity, the power output has decreased to approximately 33.7 horsepower and 39 feet head. This is a very important factor in connection

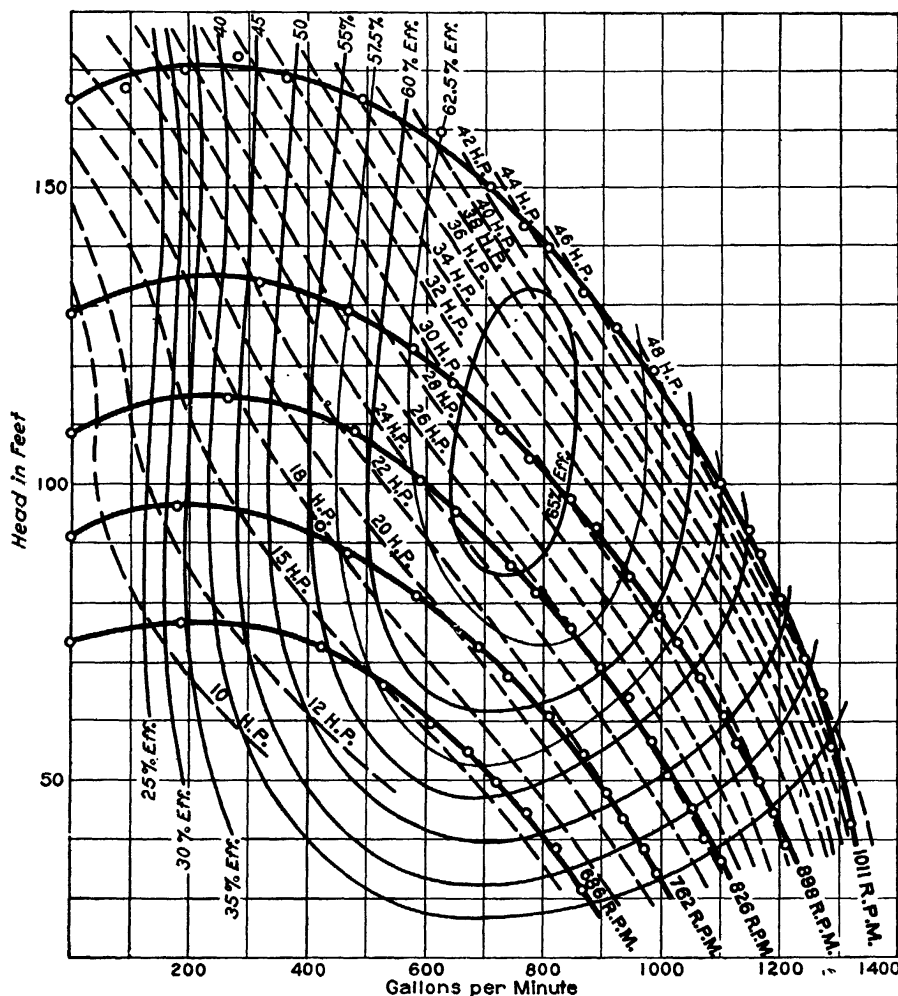


FIG. 10.—Performance curves, pump No. 1

with tests, as it shows that with a prime mover of 35 horsepower overload conditions would never exist at this speed; viz, 898 revolutions per minute. If the speed of the pump were increased as shown in Table I, new limits of capacity would exist. In any case, the pump should be operated so as to give an efficiency in excess of 50 per cent.

SPEED CURVE

The speed curve is the indicator of the proper speed at which to operate the pump to give best efficiency for a given head and capacity. This is a detail, already pointed out in this report, which must not

be neglected. Maximum speed occurs at the point of zero discharge, and in this particular instance the speed gradually lowers as the capacity of the pump increases.

ISO-EFFICIENCY CURVES

For practical use the data, first plotted in the form of characteristic curves, have been translated into the form of performance curves (figs. 10, 11, 12, 13, 14, 15, and 16).

In the selection of a pump, the pumping head is the first consideration; next is the desired capacity. For example: Assume that the pumping head is 105 feet and the desired capacity is 800 gallons per minute. With a 24-inch, 3-stage pump of the make corresponding

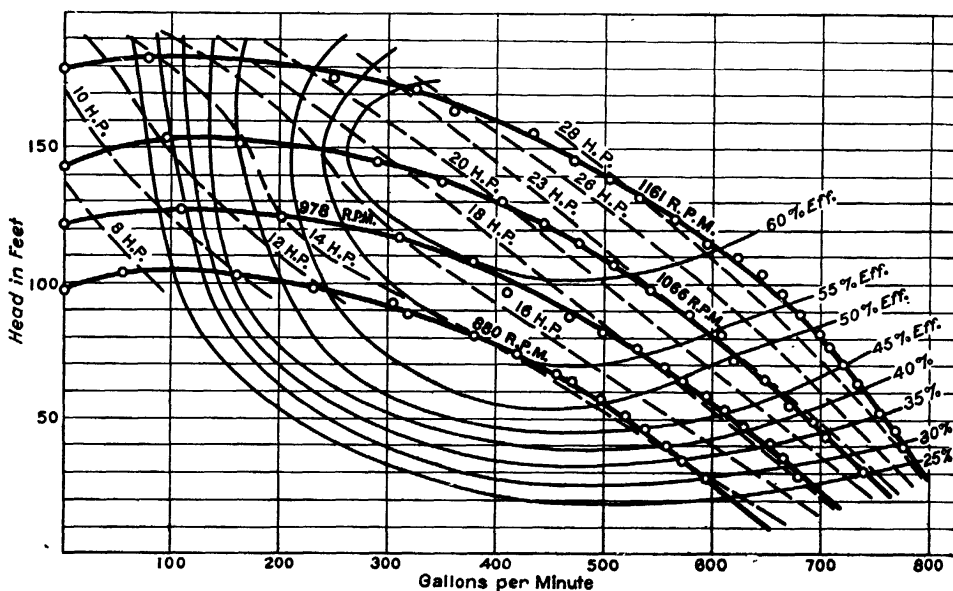


FIG. 11.—Performance curves, pump No. 2

to curves in Figures 9 and 10, an average speed of 898 revolutions per minute would give the best efficiency. At this speed a range of capacity of 500 to 1,000 gallons per minute would be possible without reducing the efficiency much below 60 per cent. For the set of conditions assumed, the power for 800 gallons per minute would be approximately 31.5 horsepower.

The curves emphasize the importance of considering the efficiency of the pump in relation to its speed. Intending purchasers should be impressed with the fact that the various types and makes of pumps have different characteristics, and that a hydraulic engineer, well versed in the performance of pumps, should be consulted before expensive pumping equipment is bought.

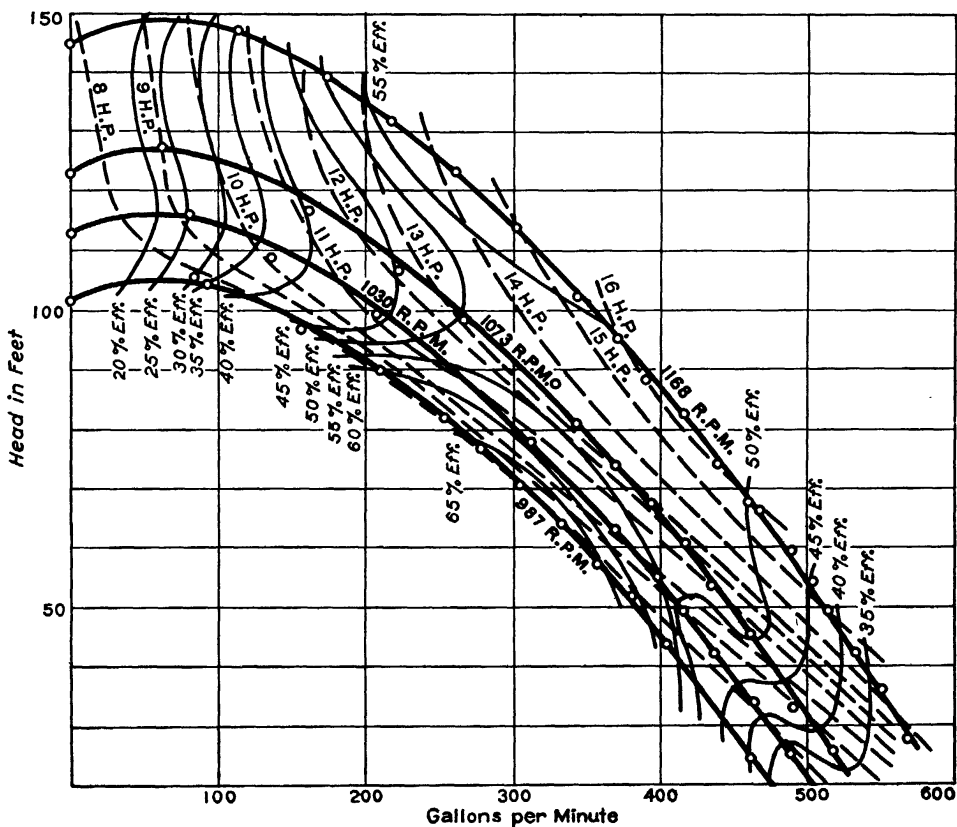


FIG. 12.—Performance curves, pump No. 3

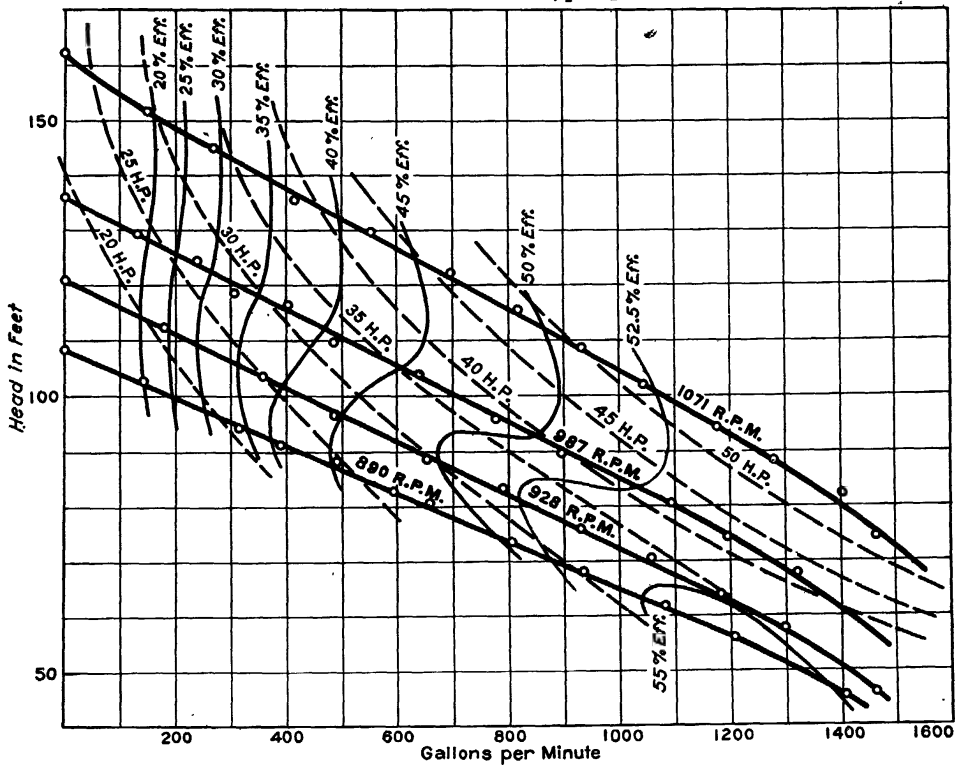


FIG. 13.—Performance curves, pump No. 4

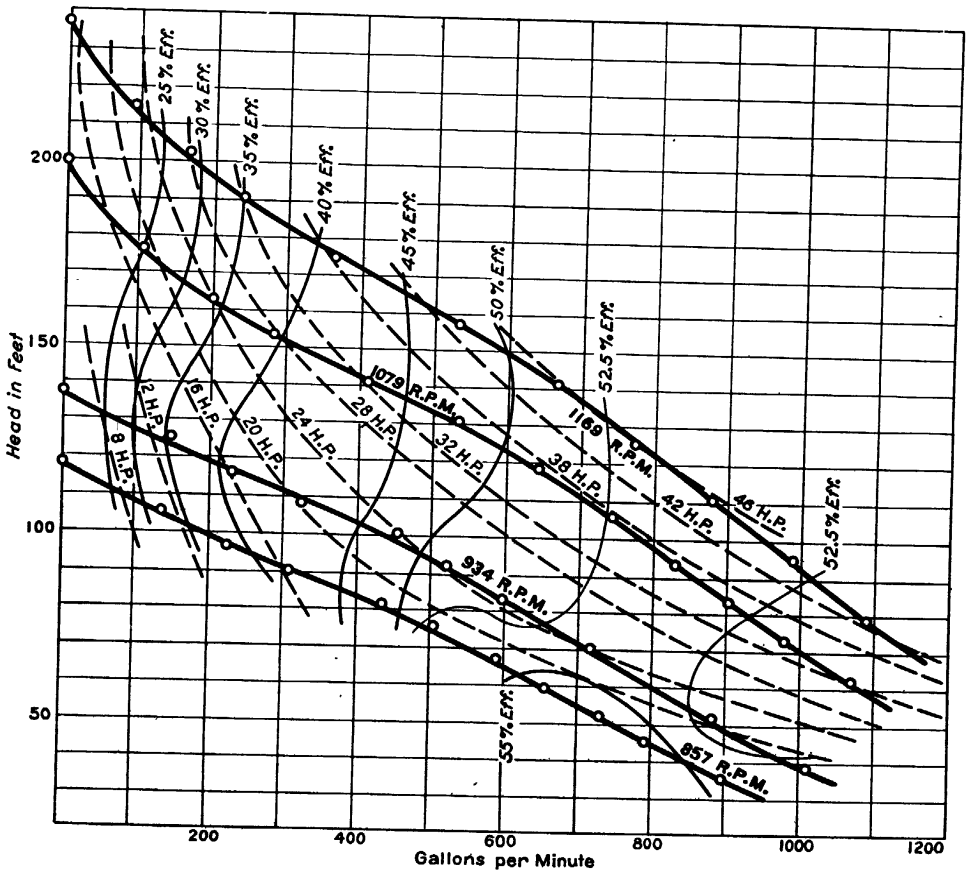


FIG. 14.—Performance curves, pump No. 5

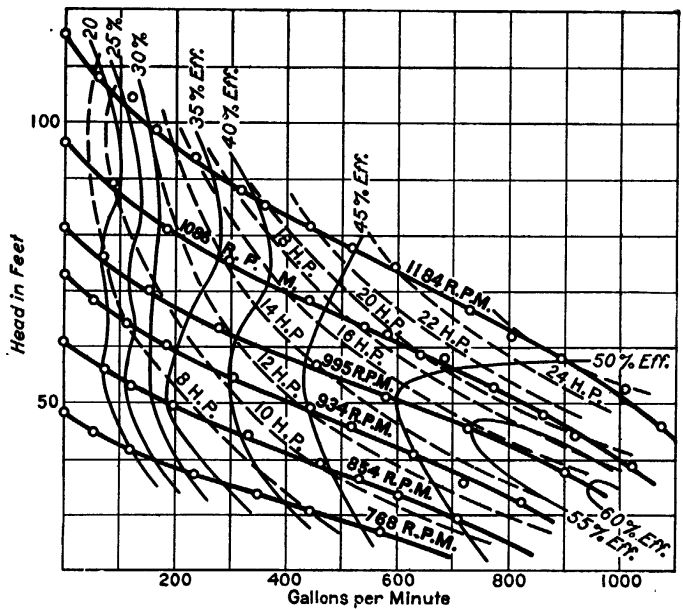


FIG. 15.—Performance curves, pump No. 6

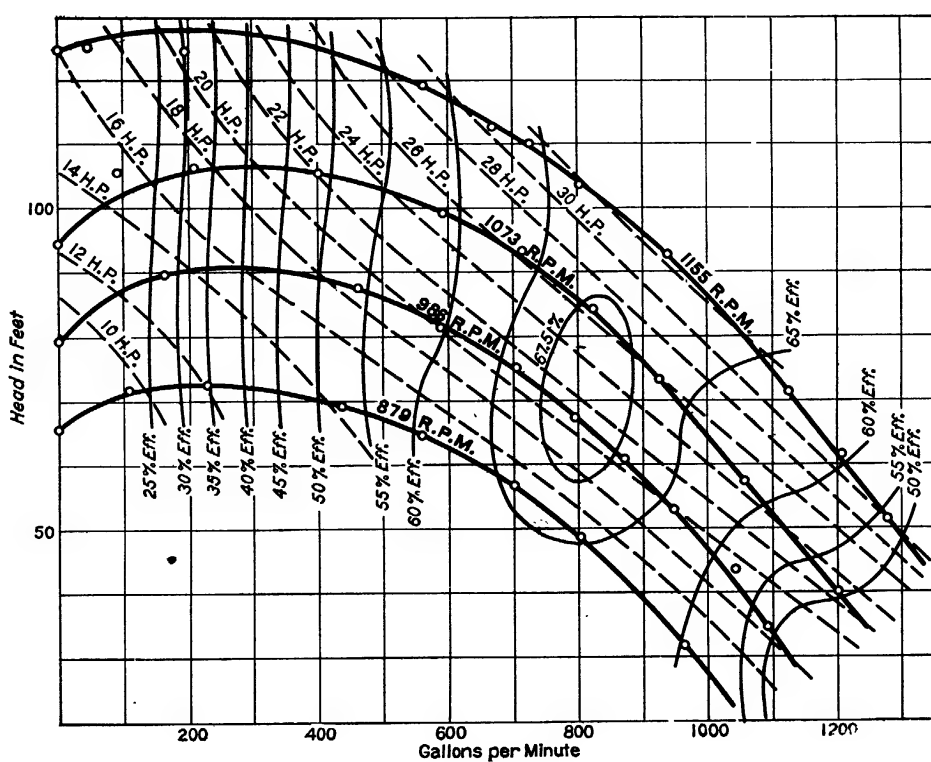


FIG. 16.—Performance curves pump No. 7

COLLOIDAL SILICA AND THE EFFICIENCY OF PHOSPHATES¹

By P. L. GILE, *Chief, Division of Soil Chemistry*, and J. G. SMITH, *Assistant Chemist, Bureau of Soils, United States Department of Agriculture*

INTRODUCTION

General fertilizer practice recognizes in a vague way that there are reactions between soils and phosphatic fertilizers which affect the efficiency of the fertilizers. Bone meal, for instance, is recommended for use on open soils which are not too dry (39, p. 169),² and for light soils not too deficient in phosphoric acid (32, p. 320). Floats are recommended by Wheeler (39, p. 173) for acid soils high in organic matter, and by Schneidewind (32, p. 321) for acid high-moor soils but not for mineral soils. Acid phosphate, according to Schneidewind (32, p. 297), is adapted to all the "better" and heavier soils. However, the extent to which a phosphate is dependent on the character of a soil is probably not sufficiently appreciated.

EFFECTS OF SOILS ON THE EFFICIENCY OF PHOSPHATES

A complicated series of reactions follows the application of a phosphate to a soil; the nature and extent of the reactions must vary in different soils; and it is to be presumed that these reactions will affect the availability or efficiency of the fertilizer. Attempts have been made, by extracting the soil with various solvents, to determine what happens to a phosphate after it is incorporated in the soil (13, 40). But it has not been possible to follow the reactions in the laboratory far enough or precisely enough to determine just how the fertilizing efficiency of a phosphate will be affected. Evidence that the soil influences the efficiency of phosphates is therefore derived chiefly from experiments with plants.

That soils affect the efficiency of the various phosphates differently is indicated by many experiments in which the fertilizing values of the soluble and insoluble phosphates are compared. It has been shown frequently that rock phosphate or bone meal may be almost as effective as acid phosphate on one type of soil but quite ineffective as compared with acid phosphate for the same crop on another type of soil. Variations in the crop increase produced by a given quantity of rock phosphate on different soils are so great that it seems quite improbable that variations in the relative efficiency of the two classes of phosphates are due in all cases to differences in their so-called "secondary" effects on the soil; that is, in decreasing acidity, supplying lime, and the like. The low efficiency of rock phosphate in quartz sand and its high efficiency in some soils indicate that in certain soils reactions take place which enhance the "availability" of the phosphate—increase the supply of phosphorus acting as a plant nutrient.

¹ Received for publication September 29, 1924; issued September, 1925.

² Reference is made by number (italic) to "Literature cited," p. 259.

While the influence of soil conditions on the comparative efficiency of insoluble and soluble phosphates has been a subject of many investigations, the influence of the soil on the absolute efficiency of a soluble phosphate has received comparatively little attention. It has been known since the early days of soil science that the phosphorus of soluble phosphates is fixed by the soil and that some of the phosphorus is available after it is fixed. But we are comparatively ignorant of how much of the "fixed" phosphoric acid is available and how much remains unavailable in different soils. The following facts indicate that in many soils a considerable part of the soluble phosphorus is rendered at least temporarily unavailable: The small proportion of the applied phosphoric acid which is recovered in the crop; the loss in efficiency of phosphates remaining in unplanted soils; and the variation in the quantity of soluble phosphate required to produce a given crop increase on different soils.

When fertilizers containing soluble forms of nitrogen or potassium are applied, not in excess, to soils responding to these ingredients, 60 to 90 per cent of the quantities applied are commonly recovered in the crop (36, 37, 23). The recovery of phosphoric acid, however, frequently amounts to only 10 to 20 per cent (26, 14). It might be held that the low recovery of phosphoric acid is due to only a part of the phosphorus being required as a plant nutrient, the remainder of the phosphorus being effective in increasing growth in other ways than in supplying available phosphorus to the plant. Some would hold, for instance, that soluble phosphates promote growth in some soils by reducing toxic, soluble aluminum in the soil (3, 2). It seems possible, however, that soluble aluminum is injurious only as it renders phosphate unavailable to the plant. The fact that relatively high recoveries of soluble phosphates may be made by plants growing in quartz sand indicate that the low recoveries from many soils are due to interaction between the phosphate and certain soil constituents (26).

Pot experiments have shown that in some soils an appreciable loss in the efficiency of acid phosphate takes place when the phosphate is applied a few weeks in advance of planting, the loss occurring in the bare soil maintained at the optimum moisture content (8). It amounted in 30 days to approximately 40 per cent of the efficiency of acid phosphate applied immediately before planting. Since a crop does not abstract much phosphoric acid per acre until it has attained some size, it is evident that there may be an appreciable loss in the efficiency of acid phosphate applied under the usual conditions.

A further indication that certain soils render soluble phosphates partially unavailable is the fact that very different quantities of acid phosphate may be needed to produce the same increased yield on two different soils, even in cases where the two soils give approximately equal increases in yield with different maximum quantities of the phosphate. In some instances this may reasonably be attributed to different weather conditions, to different cultural methods, to the use of different nitrogenous fertilizers, and the like. Variability in the response of a crop attributable to such conditions, however, can be largely eliminated in pot experiments. And a marked influence of the character of the soil on the quantity of phosphate required to produce a given crop increase is observed in pot experiments also.

Thus there is considerable evidence that the reactions taking place when a phosphate is added to a soil may increase or decrease the efficiency of different phosphatic fertilizers, and that these reactions vary in different soils. Little is definitely known about these reactions, although Liebscher (21, p. 208) and subsequent investigators have pointed out that soils high in iron and aluminum usually respond to phosphate fertilization, and many have shown that calcium carbonate influences the efficiency of the phosphates (17, 33, 34, 28, 8). Consequently, although we have a general idea of some types of soils which respond poorly to certain phosphates, we can not predict with any certainty from a laboratory examination of a soil what its effect will be on any particular phosphate. A determination of the soil constituents and the soil conditions affecting the availability of the different phosphates is obviously an essential step toward scientific fertilizer practice.

It may well be that the properties of a soil affecting the efficiency of phosphates are largely localized in the colloidal material. The absorptive and base-exchange properties of the soil, which are almost exclusively a function of the colloidal material, might be expected to influence the solubility or decomposition of the relatively insoluble phosphates. Although Gedroiz (4) has suggested that insoluble phosphates are more efficient in adsorptively unsaturated soils, the influence of the soil colloidal material on the efficiency of phosphates has not been directly studied.

The soil colloidal material is a complex substance or mixture of substances. It is quite variable in composition but is made up chiefly of silicon, aluminum, iron, and organic matter. It also contains small amounts of magnesium, calcium, potassium, sodium, manganese, titanium, phosphorus, chlorine, and sulphur (30). The condition of the elements in the colloidal material has not been definitely determined, but the constituents may be regarded as forming a mixed gel similar in nature to the artificial gels of silica, iron, alumina, etc.

Some results obtained in this laboratory indicate that a mixed gel such as occurs in soils may have certain properties not possessed by simpler gels. Nevertheless, it seemed advisable to determine the influence of relatively simple gels on the efficiency of phosphates preliminary to dealing with the colloidal material of different soils. The effects of an iron gel alone and of an alumina gel mixed with silica gel have been tested by others. Prianishnikov (27, p. 36) found that hydrous ferric oxide depressed the efficiency of bone meal markedly in a sand culture experiment with barley. In an experiment of Pfeiffer and Blanck (25), the addition of a mixture of alumina and silica gels to a sand culture depressed the quantity of phosphoric acid assimilated by yellow lupines from potassium acid phosphate. The influence of a silica gel on the efficiency of rock phosphate and acid phosphate is shown in the following experiments.

INFLUENCE OF COLLOIDAL SILICA ON PHOSPHATE EFFICIENCY, AS SHOWN BY THE GROWTH OF MILLET

It was desirable to determine the effect of colloidal silica on the efficiency of rock phosphate and acid phosphate under the simplest conditions possible. Since organic matter, other colloids, and reactive soil minerals might complicate the effect, the test was conducted in a fairly pure quartz sand (99.5 per cent silica).

The colloidal silica used in these tests was a silica gel prepared chiefly by M. S. Anderson, of the Bureau of Soils, by treating a sodium silicate solution with H_2SO_4 and HCl until a strongly acid reaction persisted. The precipitate was then washed many times until the wash water had the same reaction as the distilled water used ($\text{P}_{\text{H}}6.2$). It was then dried on the steam bath and ground to pass a 1-millimeter sieve.

A mixed gel containing silica, alumina, iron, and small quantities of lime, magnesia, and potash, was also tested in one pot for comparison with the pure silica gel. This was prepared by adding the chlorides of aluminum, iron, etc., to a sodium silicate solution in such proportions that the resulting mixture contained silica and the various bases in the same proportions as the average soil colloidal matter. The resulting gel was washed until the wash waters were free from chlorides. The washing apparently removed a considerable part of the lime, magnesia, potash, and soda, and a small part of the iron and alumina.

Golden millet (*Chaetochloa italica*) was used as the test crop. This responds markedly to phosphoric acid fertilization, and, like the other Gramineae, it has a low "feeding power" for insoluble phosphates. Glazed earthenware pots of 1 gallon capacity were used as containers. In each pot was placed 5,030 grams of sand, which was maintained at a moisture content of 8 per cent of the dry weight by the addition of distilled water, the pots being weighed daily.

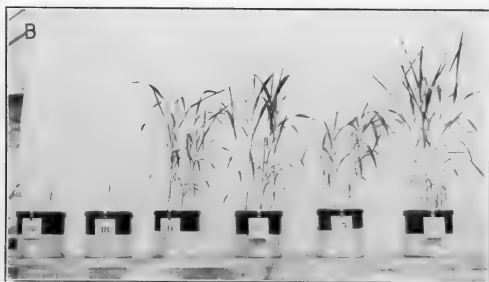
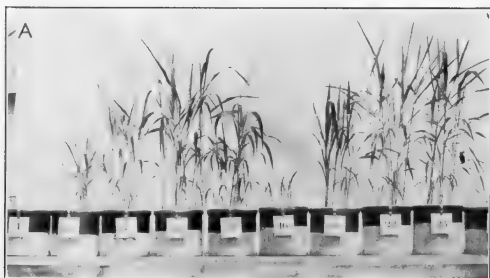
The special additions to the different pots—rock phosphate, acid phosphate, and silica gel—were thoroughly mixed with the upper half of the sand in the pot prior to planting. The rock phosphate used was a Florida pebble rock ground to pass a 100-mesh sieve. It contained 31.63 per cent of total phosphoric acid. The acid phosphate contained 18.72 per cent of available and 19.74 per cent of total phosphoric acid. Both phosphates were applied on the basis of the total phosphoric acid.

Each pot in the experiment received the following salts, applied in solution, as a basic fertilization to supply all essential nutrients except phosphorus: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1.070 grams; $(\text{NH}_4)_2\text{SO}_4$, 0.491 grams; KCl , 0.446 grams; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.914 grams; $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot \text{H}_2\text{O}$, 0.074 grams.

Seven millet plants were grown in each pot from July 29 to August 31, 1923. At the end of 33 days of growth the plants were in the joint stage, and those in the pots receiving the largest amounts of acid phosphate appeared about as large as could be grown without danger of having further growth restricted by size of pot or supply of basic nutrients. The pots were kept in a glass house and the order of the pots was shifted daily. The appearance of the plants well supplied with P_2O_5 was normal at all times (pl. 1).

The special treatments of the different pots and the oven-dry weight of the plants harvested are shown in Table I. Most of the special treatments were triplicated.

The greenhouse of the Division of Soil Fertility Investigations, Bureau of Plant Industry, was used for the conduct of this experiment.



A.—1, No phosphate; 4, acid phosphate, 0.03 gram P_2O_5 ; 7, acid phosphate, 0.06 gram P_2O_5 ; 10, acid phosphate, 0.12 gram P_2O_5 ; 13, rock phosphate, 0.24 gram P_2O_5 ; 16, no phosphate, silica gel, 50 grams; 19, acid phosphate, 0.06 gram P_2O_5 , silica gel, 50 grams; 22, rock phosphate, 0.24 gram P_2O_5 , silica gel, 50 grams; 25, rock phosphate, 0.24 gram P_2O_5 , silica gel, 150 grams

B.—3, No phosphate; 18, no phosphate, silica gel, 50 grams; 9, acid phosphate, 0.06 gram P_2O_5 ; 21, acid phosphate, 0.06 gram P_2O_5 , silica gel, 50 grams; 15, rock phosphate, 0.24 gram P_2O_5 ; 24, rock phosphate, 0.24 gram P_2O_5 , silica gel, 50 grams

TABLE I.—*Influence of colloidal silica on efficiency of acid phosphate and rock phosphate in sand, as shown by growth of millet*

Pot Nos.	Differential treatment of pots			Weight of plants above ground				Relative efficiency of the phosphoric acid (phosphoric acid of acid phosphate without silica gel=100)
	Kind of phosphate applied	Phosphoric acid (P ₂ O ₅) applied per pot	Colloidal silica applied per pot	Oven-dry weight of plants per pot			Average oven-dry weight of plants per pot	
		Gram	Grams	Grams	Grams	Grams	Grams	
1, 2, 3	No phosphate	-----	None	0.07	0.06	0.09	0.07	-----
4, 5, 6	Acid phosphate	0.03	do	1.35	1.09	1.27	1.24	100
7, 8, 9	do	.06	do	4.61	4.20	4.48	4.43	100
10, 11, 12	do	.12	do	9.17	9.56	8.53	9.09	100
13, 14, 15	Rock phosphate	.24	do	4.30	2.56	4.64	3.83	23
16, 17, 18	No phosphate	-----	50 silica gel	.32	.34	.15	.27	-----
19, 20, 21	Acid phosphate	.06	do	4.40	5.55	6.12	5.36	119
22, 23, 24	Rock phosphate	.24	do	9.79	8.61	9.99	9.46	51
25	do	.24	150 silica gel	9.10	-----	-----	9.10	49
26	do	.24	45 "mixed" gel.	.11	-----	-----	.11	0

The increase in growth with increasing quantities of P₂O₅ from acid phosphate without silica gel shows the extent to which growth was dependent upon the supply of phosphoric acid. Since the increased growth was practically a straight-line function of the quantity of acid phosphate applied, it is possible, by interpolating on the curve, to calculate, with very slight error, the growth that would have been produced by any quantity of P₂O₅ from acid phosphate below 0.12 gm. The efficiency of the P₂O₅ of rock phosphate with and without silica gel and of acid phosphate with silica gel can therefore be calculated against that of acid phosphate alone on the basis of the quantities of phosphoric acid required to produce equal increases in growth as previously suggested (?).

It is apparent that 50 grams of silica gel per pot increased the efficiency of the P₂O₅ in acid phosphate slightly, raising it from 100 to 119, while the gel more than doubled the efficiency of the P₂O₅ in rock phosphate, increasing it from 23 to 51 as compared with 100 for the P₂O₅ of acid phosphate. The 150-gram application of silica gel gave practically the same increase in efficiency of rock phosphate as the 50-gram application, within experimental error. The mixed gel containing iron and alumina completely nullified the efficiency of rock phosphate. Where no phosphate was applied the silica gel increased growth by 0.20 gram. This increase is equivalent to what would have been produced by 0.0045 gram of P₂O₅ from acid phosphate; it may reasonably be ascribed to the gel rendering the phosphorus impurities in the quartz sand, in the nutrient salts, and in the containers used more available to the plants.

The results just given indicate that colloidal silica, in a simple medium of quartz sand and nutrient salts, increases the efficiency of acid phosphate slightly and that of rock phosphate greatly. It is believed that the beneficial action of the silica gel lies in increasing the quantity of P₂O₅ in solution or the rate at which it goes into solution. A quite different explanation of the action of colloidal silica in increasing the growth of certain crops in soil and sand

cultures was recently offered by Lemmermann and Wiessmann (20). They suggested that the beneficial effect of certain forms of silica is due, not to its action on the soil, but to a direct action on the plant. This view will be discussed later.

Analyses were made of the plants where sufficient material was available, by the methods of the Association of Official Agricultural Chemists (1). The results are given in Table II.

TABLE II.—*Assimilation of silica and phosphoric acid by millet grown with acid phosphate or rock phosphate with and without silica gel*

Pot Nos.	Differential treatment of pots	In the oven-dry substance of the plants			In the pure ash		Present in 7 plants (from 1 pot)		Of total P_2O_5 supplied, percentage present in plants above ground
		Pure ash, C , CO_2 , and sand free	Silica (SiO_2)	Phosphoric acid (P_2O_5)	Silica (SiO_2)	Phosphoric acid (P_2O_5)	Silica (SiO_2)	Phosphoric acid (P_2O_5)	
7, 8, 9.....	0.06 gram P_2O_5 , acid phosphate.....	P. ct. 9.27	P. ct. 0.96	P. ct. 0.40	P. ct. 10.33	P. ct. 4.07	Gm. 0.0426	Gm. 0.0177	P. ct. 29.5
10, 11, 12.....	0.12 gram P_2O_5 , acid phosphate.....	6.48	.59	.47	9.14	7.34	.0536	.0427	35.7
13, 14, 15.....	0.24 gram P_2O_5 , rock phosphate.....	15.09	4.39	.59	29.05	4.02	.1681	.0226	9.4
19, 20, 21.....	0.06 gram P_2O_5 , acid phosphate, 50 grams silica gel.....	9.66	2.54	.43	26.34	4.49	.1361	.0230	38.3
22, 23, 24.....	0.24 gram P_2O_5 , rock phosphate, 50 grams silica gel.....	8.78	3.08	.54	35.10	6.02	.2914	.0511	21.3
25.....	0.24 gram P_2O_5 , rock phosphate, 150 grams silica gel.....	8.40	2.74	.57	32.47	6.55	.2493	.0519	21.6

The percentages of phosphoric acid in the dry substance generally increased with the growth. However, the plants grown with rock phosphate contained a somewhat higher percentage of phosphoric acid in the dry substance than the plants grown with acid phosphate, although they contained approximately the same percentage of phosphoric acid in the ash as the acid-phosphate plants of equivalent growth. The percentage of phosphoric acid in the plant is doubtless influenced to some extent by the rates at which the plants grew during different stages of their development, or by slightly different stages of maturity at the time of harvesting.

During the latter part of the growing period the rock-phosphate plants appeared to be picking up slightly on the acid-phosphate plants; owing to increased excretion of carbon dioxide or to some other cause, they doubtless obtained phosphorus at a somewhat faster rate toward the end of the growing period than at the start. The assimilability of the phosphorus of acid phosphate, on the other hand, might have been more constant. Such differences would account for the different percentages of P_2O_5 in the dry substance of the plant.

Addition of silica gel increased the percentage of silica in the acid-phosphate plants by more than one and a half times. In the case of the rock-phosphate plants, however, the silica gel diminished the percentage of silica in the dry substance and raised the silica in the ash relatively little.

The quantities of phosphoric acid and silica present in seven plants (the yield from one pot) are significant in indicating how silica gel increased the yield. It will be noted that the quantities

of silica removed by the crop bear little or no relation to the yields of the differently treated pots given in Table I. The quantities of phosphoric acid removed, however, approximate fairly closely the relative yields produced by the various treatments. It would thus appear that the silica in the plant was not a determining factor in the yield and that the phosphoric acid assimilated probably was the limiting condition of growth.

The last column in Table II shows that the application of silica gel promoted the assimilation of phosphoric acid from rock phosphate to a marked extent, while it increased the assimilation of phosphoric acid from acid phosphate comparatively little.

In a complex medium such as a soil, silica gel might promote the availability of rock phosphate without the increased availability being apparent through any test except that of the growing crop. However, in the simple medium of this experiment—quartz sand and soluble salts—an increased availability of rock phosphate should be revealed by an ordinary chemical analysis, since the quartz sand, except for slight impurities of iron and possibly other substances, would be without action on phosphoric acid, brought into solution. Laboratory tests were conducted on the solubility of rock phosphate simply with the nutrient salts used in the basic fertilization.

EFFECT OF COLLOIDAL SILICA ON THE DECOMPOSITION OF ROCK PHOSPHATE

The concentration and relative proportions of the nutrient salts added to the pots (basic fertilization) in the experiment just described of course underwent marked alteration with the growth of the plants. It was impossible, therefore, to determine the solubility or decomposition of rock phosphate in a solution representative of that present in the pots during all stages of the growth of the crop. However, the effect of silica gel in promoting the growth of plants supplied with rock phosphate was apparent when the plants were only a few inches high, and at this time the composition of the nutrient salts must have been essentially the same as at the start of the experiment. It was not expected that the quantities of phosphoric acid found in solution on shaking up rock phosphate with the nutrient salts and silica gel would correspond quantitatively to the quantities present in the pots at all times, but it was thought that the results obtained would indicate qualitatively the effect of silica gel on the solution or decomposition of rock phosphate in the pots.

In the first test, 0.5 gram of rock phosphate or 0.3 gram of acid phosphate was shaken up with a mixed salt solution or with a salt solution plus silica gel. The salt solution contained the equivalent of 1.25 grams of anhydrous salts in 250 cubic centimeters of water, the same salts being present in the same proportions as in the mixture used for the basic fertilization of the pot experiment. The silica gel added was the same as that used in the pot experiment. The mixture was shaken at intervals during seven days standing. It was then filtered through a Pasteur-Chamberland filter and P_2O_5 was determined in an aliquot of the filtrate, which was perfectly clear. Prior to precipitating the phosphorus, special precautions were taken to rid the filtrate of silica which might contaminate the phosphorus precipitate. Three dehydrations with HCl were made with intervening filtrations. The P_2O_5 was determined as $Mg_2P_2O_7$. The

quantities of P_2O_5 and SiO_2 found in solution,³ calculated for the 250 cubic centimeters of solution, are shown in Table III, together with the special treatments of each flask.

TABLE III.—Soluble phosphoric acid obtained from rock phosphate and acid phosphate in a mixed salt solution with or without silica gel

Contents of flasks (in addition to 1.25 grams mixed salts and 250 cubic centimeters water)	Phosphoric acid (P_2O_5) in solution in 250 cubic centimeters	Silica (SiO_2) in solution in 250 cubic centimeters
	Gram	Gram
0.5 gram rock phosphate.....	0.0010	0.0021
0.5 gram rock phosphate, 5 grams silica gel.....	.0012	.0184
0.5 gram rock phosphate, 15 grams silica gel.....	.0019	.0207
0.5 gram rock phosphate, 30 grams silica gel.....	.0045	.0229
0.3 gram acid phosphate.....	.0344	.0025
0.3 gram acid phosphate, 15 grams silica gel.....	.0346	.0231

The 30-gram addition of silica gel appears to have markedly increased the quantity of phosphoric acid brought into solution from rock phosphate.⁴ The increase produced by 15 grams of gel also appears real, but that attributable to the 5 grams of gel is probably less than experimental error. The solubility of the acid phosphate P_2O_5 in the salt solution was apparently not affected by the silica gel.

In order to check the accuracy of these results, a second test, similar to the first, was conducted with the rock phosphate. The method and materials of the second test were the same as those of the first, but the quantities of phosphate, gel, and salts were somewhat different; also most of the different treatments were duplicated. One flask (the last in the table) was included in which the mixed salts were varied solely by omitting the iron. The mixtures were allowed to stand 12 days with an occasional shaking. The results of this second test are given in Table IV.

TABLE IV.—Soluble phosphoric acid obtained from rock phosphate in a mixed salt solution with or without silica gel

Contents of flasks (in addition to 1.64 grams of mixed salts and 251 cubic centimeters water)	Phosphoric acid: (P_2O_5) in solution in 251 cubic centimeters		Silica(SiO_2) in solution in 251 cubic centimeters	
	Individual flasks	Average of duplicates	Individual flasks	Average of duplicates
	Gram	Gram	Gram	Gram
No phosphate, 31.3 grams silica gel.....	{ 0.0011 .0006 }	0.0009	{ 0.0228 .0187 }	0.0208
Rock phosphate containing 0.15 gram P_2O_5	{ .0006 .0005 }	.0006	{ .0023 .0021 }	.0022
Rock phosphate containing 0.15 gram P_2O_5 , 31.3 grams silica gel.....	{ .0054 .0054 }	.0054	{ .0237 .0227 }	.0232
Rock phosphate containing 0.15 grams P_2O_5 , 31.3 grams silica gel, no Fe in mixed salts.....	.0067	-----	.0215	-----

³ The quantity of silica shown as "in solution" represents the quantity passing through the Pasteur-Chamberland filter. Part or all of this may have been present as a very finely dispersed sol rather than as a true solution.

⁴ Similar results were obtained by Mattson (24, p. 77) on digesting a precipitated tricalcium phosphate with water suspensions of sphagnum peat, ceramic clay and quartz flour. The first two substances were particularly effective in decomposing the phosphate.

This test confirms the preceding one in showing that when rock phosphate is exposed to a mixed salt solution the addition of a fairly large quantity of silica gel markedly increases the quantity of phosphoric acid in solution. The omission of the small quantity of iron from the mixed salts apparently increases the quantity of phosphoric acid in solution. Doubtless the iron precipitates some of the phosphoric acid brought into solution by the action of the colloidal silica.

DISCUSSION OF RESULTS

The yields of plants in the pot experiment, where the phosphorus supply was the limiting factor, the quantities of phosphoric acid in the plants, and the laboratory determinations of the solubility or decomposability of the phosphates, all indicate that silica gel under the simple conditions of these experiments markedly increased the assimilability of the phosphoric acid in rock phosphate and influenced relatively little the assimilability of the phosphoric acid in acid phosphate. If the plant experiment had been conducted in a different medium or with a different basic fertilization, it is quite possible that the silica gel would have had a more or less marked effect on these two phosphates.

Lemmermann and Wiessmann (20) conducted many pot experiments in which a silica gel and other finely divided forms of silica were added to quartz sand and to soils. The phosphorus supply was obviously the chief factor limiting growth in these experiments and in all cases the silica gel increased the yield appreciably. As a result of their experiments Lemmermann and Wiessmann concluded that silica acts to a certain degree as a substitute for phosphoric acid (it is "phosphorsäuresparend") and that the action is within the plant, not on the soil. While the results of these experiments are not questioned, it is believed that the conclusion drawn is hardly justified, especially in view of the fact that the plants were not analyzed for either silica or phosphoric acid. The results obtained by these investigators could be explained either by assuming that silica acted as a substitute for phosphoric acid in the plant or that silica gel rendered the phosphorus in the medium more available.

The fact that plants which normally contain a high percentage of silica have, in certain experiments, been grown practically free from silica shows that silicon is not an essential nutrient such as nitrogen, phosphorus, or potassium. It is evident that almost all the silica assimilated by plants can be omitted without abnormally affecting growth.⁵ Oats, the crop grown by Lemmermann and Wiessmann in most of their experiments, usually contain about 50 per cent of silica

⁵ Sachs (31, p. 288) and Knop (18, p. 301) grew maize, and Kreuzhage and Wolff (19) grew oats, in nutrient solutions lacking silica, the plants containing only a fraction of the normal content of silica. Jodin (16) grew maize for four generations in this way. Rice, which normally contains about 10 per cent of silica in the dry matter (5) contained only 0.2 per cent of silica grown in a silica-free nutrient solution (6, p. 508). However, owing to experimental difficulties, it has not been shown whether good plants can be grown absolutely devoid of silica.

Several investigators have obtained increased growth by adding silica in some form to nutrient solutions or to the soil (19, 29, 11, 12). None of these experiments established that the increased growth was due to an increased assimilation of silica. The nutrient solution with and without silica used by Rautenberg and Kühn were of different compositions. Apparently none of the plants in Grégoire's experiments made a vigorous growth, and an increase was also obtained with alumina. The data considered by Hall and Morison were obtained from a field plot that had been treated with sodium silicate, an alkaline substance. The work of Livingston (22) and Jennings (16) indicate that growth may be altered in nutrient solutions by nonnutrient substances. While it has not been proved that plant growth is promoted by assimilation of silica, it is also yet to be proved that assimilation of silica does not promote growth.

in the ash of the whole plant (38). If growth is not abnormally diminished by the omission of most of this silica, it is hard to conceive of growth being promoted by a relatively small increase in silica over the average content.

It should be noted that Lemmermann and Wiessmann obtained significant increases only with pure silica gel, which is adsorptively active and contains no ions for base exchange. Kieselguhr, kaolin, various finely powdered silicas, and permutite, which contains exchangeable calcium, gave no increase. In view of the hypothesis they proposed, it would have been highly advisable to have determined which forms of silica increased the silica contents of the plants.

Experimental results reported in this paper confirm certain results of Lemmermann and Wiessmann, but contradict their hypothesis of the beneficial action of silica gel. Data given in Table I and II indicate that an increased assimilation of silica by the plant does not enable the plant to get along with less phosphoric acid.

Addition of silica gel to pots receiving 0.06 gram P_2O_5 from acid phosphate increased the growth of plants by 21 per cent, the quantity of P_2O_5 taken up by 30 per cent, and the quantity of silica assimilated by 219 per cent. If we attribute the slight increase in growth to the slight increase in the quantity of phosphoric acid assimilated the 219 per cent increase in silica taken up had no effect on growth. Plants grown with 0.24 gram P_2O_5 from rock phosphate made 14 per cent less growth than plants grown with 0.06 gram P_2O_5 from acid phosphate, although they took up 28 per cent more phosphoric acid and 295 per cent more silica. Here the increased assimilation of silica would seem to have diminished rather than enhanced the effectiveness of the phosphoric acid in the plant. Plants grown with 0.24 gram P_2O_5 from rock phosphate plus silica gel made 4 per cent more growth than plants grown with 0.12 gram P_2O_5 from acid phosphate and assimilated 20 per cent more phosphoric acid and 444 per cent more silica. In this case the slight increase in growth is more than accounted for by the phosphoric acid assimilated and no beneficial action of the 444 per cent excess assimilation of silica is apparent.⁶

While it seems very apparent that the addition of silica gel to the quartz sand and salts of our pot experiment increased the assimilability of the phosphoric acid of rock phosphate, a complete explanation of the action of the gel can hardly be offered on the basis of the work done. The laboratory tests with solutions indicate that the silica gel increased the assimilability of rock phosphate by increasing the quantity of phosphoric acid in solution. Possibly the gel increased the decomposition of the rock phosphate by adsorbing calcium hydroxide—one of the end products of the decomposition. It is also possible that the gel acted by adsorbing OH ions.⁷ There was some evidence of this in tests made of the hydrogen-ion concentrations of the solutions in the different flasks. However, the water extracts of the various pots after the plants had been grown in them did not bear this out.

⁶ It might be held that failure of the enhanced assimilation of silica to increase growth in this case was because growth was no longer limited by the phosphorus supply. However, the steep form of the curve obtained on plotting growth increase against 0.03, 0.06, and 0.12 gram P_2O_5 from acid phosphate would indicate that this was not the case.

⁷ The experiments of Gordon and Starkey (10, 35) indicate that silica gel does not absorb an appreciable amount of Ca from neutral salts, especially from an acid solution, although alumina and iron gels may. According to Glixelli (9), silica gel absorbs OH ions and the hydroxides of Na, K, Ca, and Ba.

The action of silica gel in the simple medium of these experiments probably does not correspond closely to the action of the colloidal material which is present in a soil. The results do show that a substance of the same general physical, but not chemical, nature as the soil colloidal material may markedly increase the efficiency of a phosphate fertilizer. Previous studies have shown that other substances of the same general physical nature as the soil colloidal material (an iron gel and an alumina gel acting with a silica gel) may appreciably depress the efficiency of phosphate fertilizers. As previously mentioned, the soil colloidal material is a mixed gel containing many different elements but consisting chiefly of silica, iron, and alumina. It might be supposed that such a mixed gel would have an action on phosphates approaching that of a silica gel. Apparently this is not necessarily so. The mixed gel used in these experiments depressed the efficiency of rock phosphate to zero, which is all that would be expected of a pure iron gel. At first thought this seems rather surprising. It should be borne in mind, however, that while a pure iron or alumina gel has somewhat of an adsorptive power for electrolytes, it does not give an appreciable concentration of iron or alumina in solution when treated with a neutral salt. On the other hand, it was found by Anderson, of this laboratory, that the mixed gel which he had prepared gave a heavy concentration of iron and alumina in solution when treated with a fairly concentrated potassium chloride solution. The depressing action of the mixed gel in the writers' experiments was therefore probably due to the maintenance of sufficient iron and alumina in solution to preclude the presence of phosphate ions.

SUMMARY

In an experiment with millet grown in sand culture, the addition of silica gel greatly increased the growth of plants supplied with rock phosphate and only slightly increased the growth of plants supplied with acid phosphate. The addition of a mixed gel containing iron, alumina, and silica to a pot receiving rock-phosphate was marked by reduced growth as compared with the growth made in pots receiving no phosphoric acid.

Growth made by the plants in the different pots was approximately proportional to the quantities of phosphoric acid in the plants, but seemed to bear no relation to the quantities of silica taken up.

The beneficial action of silica gel on the growth of plants supplied with rock phosphate is ascribed to the increasing of the availability of this phosphate through increasing the quantity of phosphoric acid in solution. Similarly, the deleterious effect of the mixed gel is ascribed to a diminishing of the quantity of phosphoric acid in solution, owing to soluble iron and aluminum produced by base exchange.

This conclusion is substantiated by the quantities of phosphoric acid found in solution on shaking up rock phosphate with silica gel and a nutrient solution such as was used in the pot experiment.

Criticism is made of the idea that the increased growth of plants observed in similar experiments is due to an increased assimilation of silica which enables the plant to get along with less phosphoric acid.

The relation of the action of silica gel on the efficiency of phosphates to that of the soil colloidal material is discussed.

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BEST TIME FOR SOWING SILVER FIR IN THE NURSERY ¹

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INTRODUCTION

Early attempts to grow silver fir (*Abies grandis*) at the Wind River Nursery, Carson, Wash., resulted in almost complete failure. One exception, however, was a successful sowing in the late spring. Since the previous losses were nearly all due to damping off, this singular success was attributed to the later date of sowing and the inactivity of the damping-off fungi after the soil had warmed. In order to correlate successful germination with time of sowing and soil temperature, the following experiment was undertaken.

METHOD

The unit seed bed area used was one-half of a standard 4 by 12 foot bed, making the unit 4 by 6 feet. The first bed was sown on November 16; the second on March 25 of the following spring, the earliest date it was possible to work the soil. The series was then continued at about 10-day intervals.

The seed used was gathered in the fall just before the first sowing, at Red Mountain, on the Columbia National Forest, at about 4,000 feet elevation. It was extracted by air drying where gathered, and was then shipped to the Wind River Nursery and stored in sacks in a cool, dry room. A cutting test on November 15 showed the seed to be 39 per cent good, 10 per cent wormy, and 51 per cent bad. Another cutting test March 21 following showed 36 per cent good, 12 per cent wormy, and 52 per cent bad. There were 12,550 seeds per pound. Sowing was at the rate of 2,500 good seeds per bed, based on weight and cutting test.

The fall-sown bed remained unshaded, as did the spring-sown beds, until the middle of June, when 50 per cent shade was applied. The shades were left off to prevent damping off and to produce a warmer soil in the seed beds.

The seed beds were not allowed to become dry but the watering was done at a time when the surface would dry soon after, to prevent damping off.

Soil-temperature readings were taken at 1-inch and 6-inch depths at intervals of about three days.

Germination, mortality, and survival counts were taken daily from April 17, one week before germination began, until August 15, the close of germination. These counts were made in three areas in each bed, each area being 2 feet by 6 inches. Hence 3 square feet were counted. These areas were permanently marked with pegs and cord at the time of sowing. The seedlings were marked with colored toothpicks, each color indicating a one-week age class. Causes of mortality were determined by cultures from dead or dying seedlings.

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Notes on terminal-bud formation and on second growth were taken September 3 and November 6.

After the close of the growing season weights and measurements of the top and root development of the seedlings, taken from 25 plants arbitrarily selected from each bed, were recorded.

RESULTS

A general correlation of soil temperature and germination of seeds is established. When the soil temperatures given in Figure 1 and the period of best germination in Table I are related, definite relations between soil temperature and germination are seen, although specific instances differ. The periods of best germination for the sowings of March 25 and April 4 are shown to be later than those for the sowings of April 13 and April 25. This condition emphasizes the danger of early spring sowing because of the long dormant period of the seed and the consequent exposure to damping off. The month of May was unfavorable to germination, and the periods of best germination were after the soil had warmed. Likewise the best germination period of the fall sowing came when the soil was warmest in the latter part of April and early May. The general indications of the spring sowings are that germination does not proceed at its best until the soil temperature is 70° F. or warmer to a depth of 6 inches. Surface temperature may be higher, but a cooler soil below the surface causes rapid and extreme fluctuations at the surface and the seed does not get the benefit of a high enough temperature for good germination.

The fall sowing resulted in the greatest germination and also the highest percentage of survival, as shown by Table I and Figures 2 and 3.

TABLE I.—Effect of time of sowing on germination and bud maturity

Time of sowing	Number of days before germination	2-week periods of best germination	Total germination per square foot	Percentage of buds mature	
				Sept. 3	Nov. 6
			Number	Per cent	Per cent
Nov. 16, 1917.....	At least 30 days in spring.	Apr. 23–May 6.....	52.5	88	100
Mar. 25, 1918.....	73	June 20–July 3.....	20.8	73	100
Apr. 4, 1918.....	64	June 21–July 4.....	28.2	85	100
Apr. 13, 1918.....	58	June 17–June 30.....	24.9	71	100
Apr. 25, 1918.....	53	do.....	23.8	23	100
May 4, 1918.....	51	June 24–July 7.....	20.1	24	100
May 20, 1918.....	35	do.....	28.2	6	98
May 31, 1918.....	38	July 15–July 28.....	27.2	11	95

The losses were due almost entirely to damping off, practically all those indicated by the cultures being caused by *Pythium debaryanum*. *Rhizoctonia* sp. was found in a few cultures later in the season.

Figure 2 shows that germination proceeded quite uniformly in each bed after it began, with the exception of the fall sowing. Although weekly records vary, all of the spring sowings were quite uniform in final germination, with slightly wider differences in survival, as shown in Figure 3. The dormant period of the seed in the seed bed was very much shorter in the later sowings. This is an important factor, as it reduces the period of exposure to damping off. The fall-

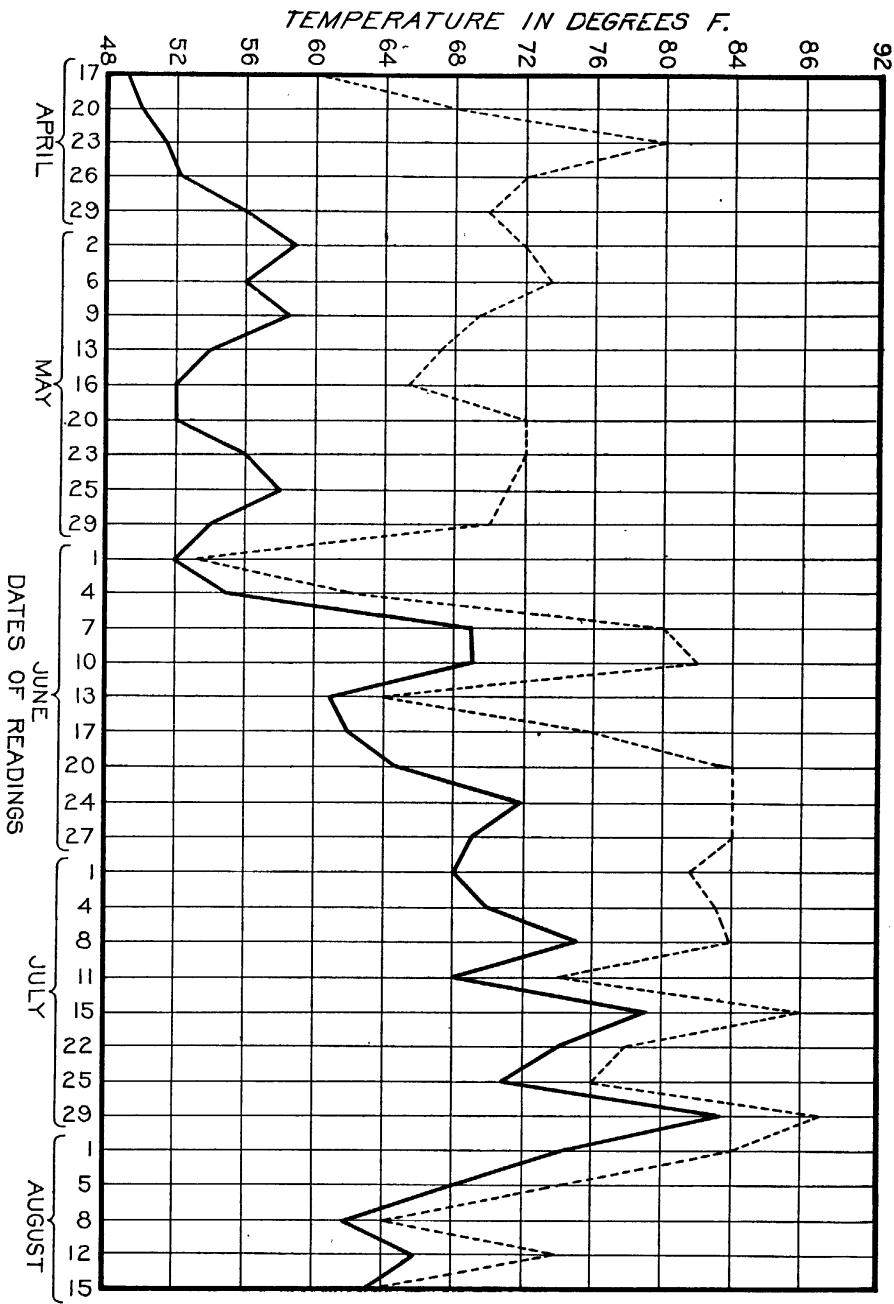


FIG. 1.—March of soil temperature at depths of 1 and 6 inches in the nursery, 1918

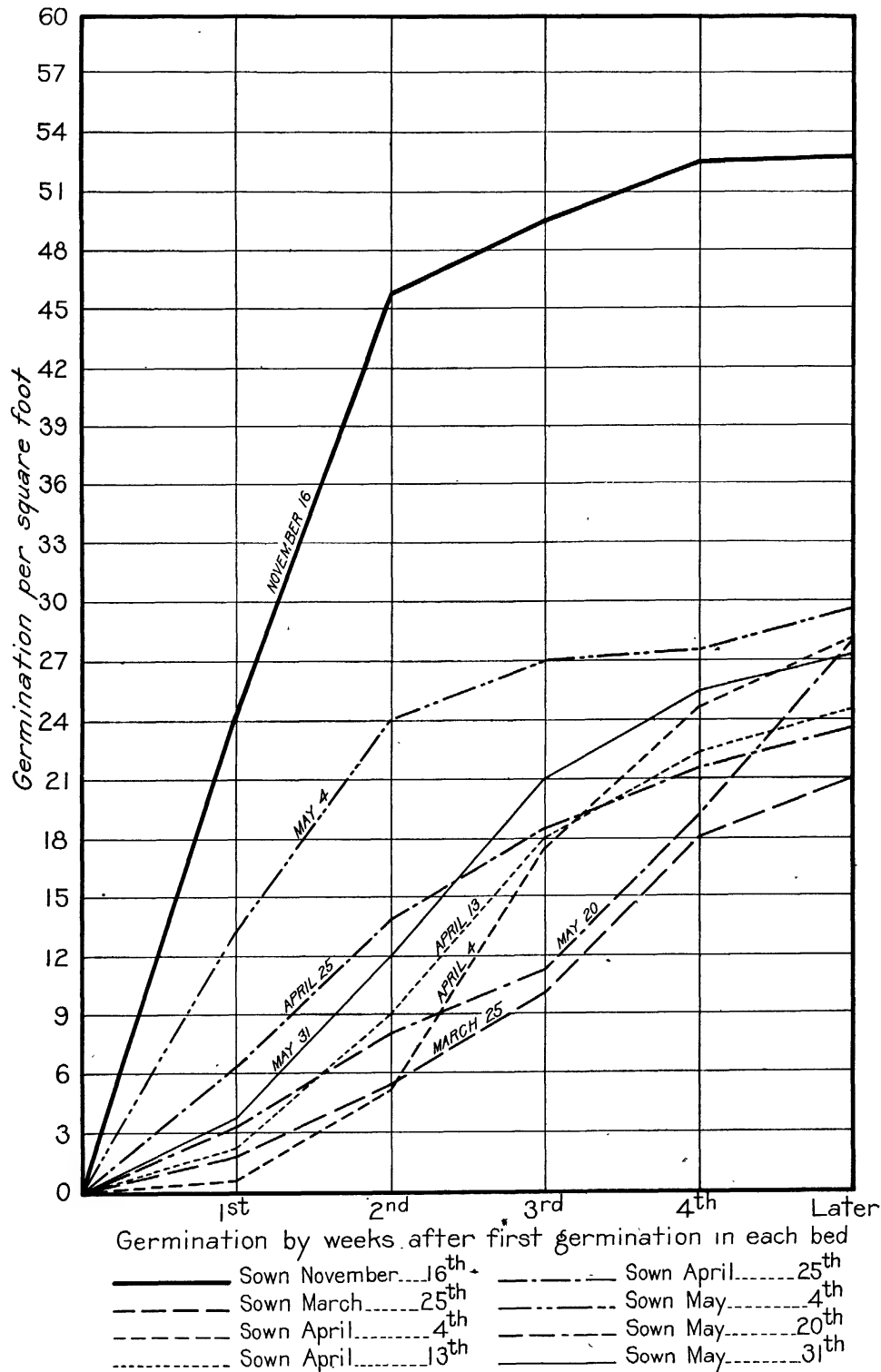


FIG. 2.—Germination of silver fir seed by weeks after first germination in each bed

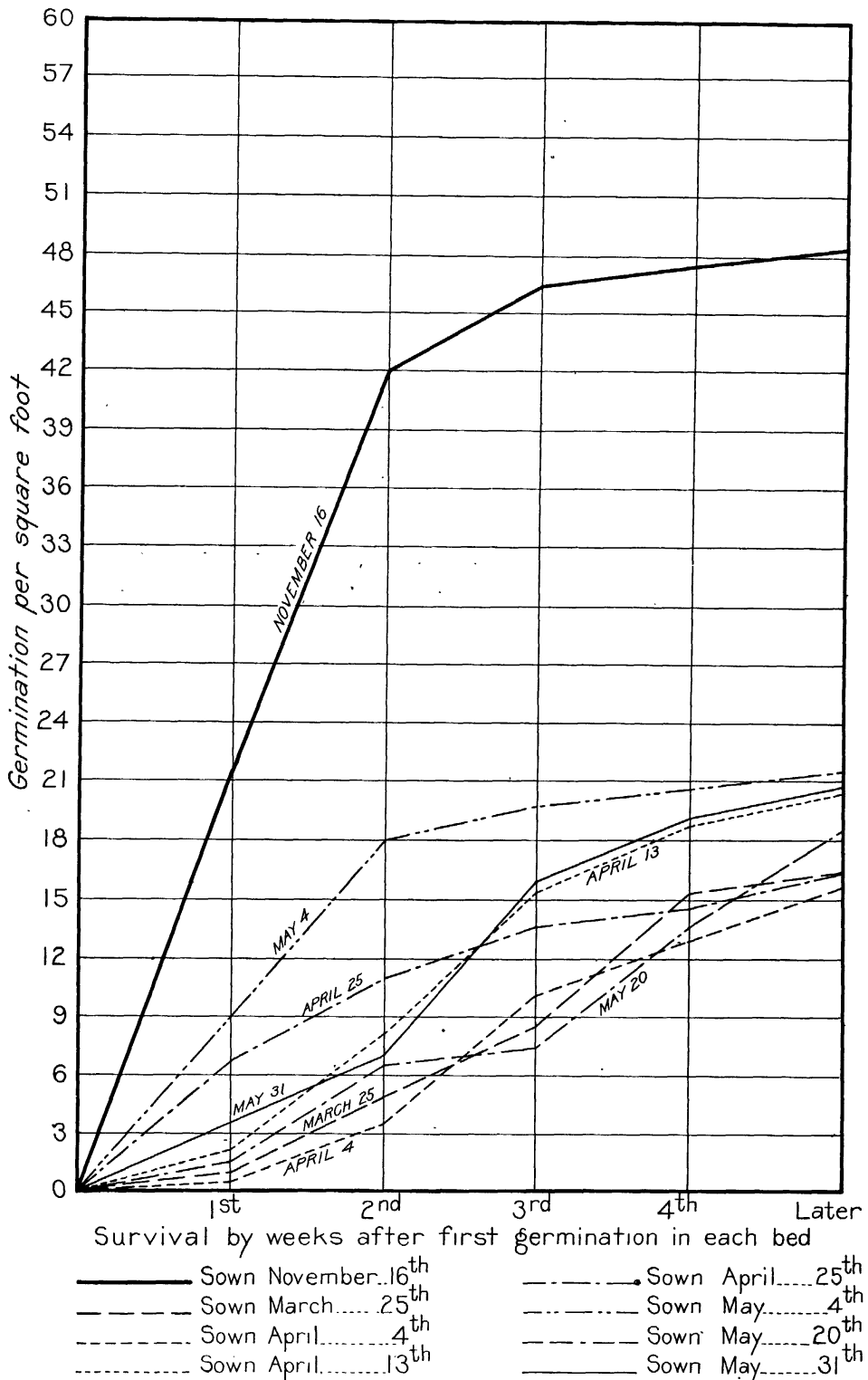


FIG. 3.—Survival of silver fir seedlings by periods in weeks following the first germination, fall and spring sowings.

sown seedlings matured their buds earlier, although this season the buds of all of the seedlings matured. The first killing frost came in November, while usually the first frosts occur in early October. In an average fall the buds of most of the spring-sown seedlings would have been immature and subject to injury by frost. There was no tendency to develop second growth in any of the stock.

TABLE II.—*Effect of time of sowing on development of seedlings*

Time of sowing	Weight of entire root system	Weight of root system pruned to 5-inch length	Secondary roots	Length of root	Weight of top	Length of top
	Ounces	Ounces	Number	Inches	Ounces	Inches
Nov. 16, 1917.....	0.56	0.52	11	6.3	0.60	1.4
Mar. 25, 1918.....	.28	.28	2	3.8	.48	1.0
Apr. 4, 1918.....	.36	.36	4	5.6	.44	1.0
Apr. 13, 1918.....	.28	.28	2	4.1	.48	1.1
Apr. 25, 1918.....	.24	.24	2	4.8	.48	1.3
May 4, 1918.....	.24	.24	1	3.9	.40	1.0
May 20, 1918.....	.20	.20	1	4.2	.36	1.0
May 31, 1918.....	.16	.16	1	2.3	.28	1.0

The fall sowing produced by far the best developed plants, as shown in Table II. The plants were larger, more sturdy, and had a well-developed, branching root system. The plants of the spring sowings showed only normal variations, except the late sowings of May 20 and May 31. The uniform development of all of the other sowings was no doubt due to the delayed germination of the earlier sowings and the optimum germination of all of these sowings at approximately the same time.

SUMMARY

The results of this experiment show that the best developed and hardiest plants result from fall sowing. Simply on the basis of plant development fall sowing would be recommended, but the danger of damping off complicates the question. There is always the possibility of loss by damping off. Spring sowing should be done not earlier than the last week of April and not later than the second week in May, in an average season.

GIRDLING AS A MEANS OF REMOVING UNDESIRABLE TREE SPECIES IN THE WESTERN WHITE PINE TYPE¹

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INTRODUCTION

Western hemlock (*Tsuga heterophylla*) and white or grand fir (*Abies grandis*), two shade-tolerating species which are found in more or less abundance in the western white pine type in northern Idaho and northwestern Montana, must usually be classified as undesirable species silviculturally, and for several reasons. In both species the liability to infection by heart-rot at an early age causes such a high percentage of defect that by the time the trees reach merchantable or saw-log size they are apt to be largely or entirely worthless. Even when sound, the material produced by these species is of poor quality and low value; and in stands in which they occur to any extent the stumpage yield per acre is materially decreased by the fact that they occupy space which might support more valuable species.

A particularly undesirable feature is the dense shade cast by these trees. The advance growth of hemlock and white fir seedlings and saplings which comes in under the mature trees has a tendency to monopolize the openings between the larger trees and to repel less shade-tolerant species. As a result, on cutting areas where seedlings, saplings, and larger trees of these species are present, it is difficult to obtain establishment and growth of reproduction of the more intolerant desirable species of western white pine (*Pinus monticola*) and western larch (*Larix occidentalis*).

In order to get maximum production of the most valuable species in future stands, the hemlock and white fir trees left standing on cut-over areas should be disposed of, and measures to prevent or hinder the reseeding of the ground by these species should be taken.

Possible methods of disposing of undesirable tree species include girdling, burning, and poisoning.

Killing trees by fire is particularly effective with thin-barked species, such as hemlock and white fir. Fire may be applied by burning piles of forest débris at the base of undesirable trees, or in thickets; or it may be applied as broadcast burning of slashings, which destroys most or all of the undesirable reproduction and larger trees. Broadcast burning, however, in most cases destroys also the valuable seed trees and trees left for increased growth, as well as most or all of the seed stored in the surface duff.

Poisoning, or introducing some toxic material into the tree through the sap, has been used to some extent for killing hardwood stump sprouts in farm clearings in the Eastern States. Although the poisoning method may very often be too expensive to be practicable, it offers possibilities worthy of investigation.

¹ Received for publication Oct. 28, 1924; issued September, 1925.

Girdling, an old and common method, consists of so severing the cambium and phloem of the tree that the nutritive communication between root and crown is permanently interrupted.

EARLY ATTEMPTS AT KILLING BY GIRDLING

As far as known to the writers, the first attempt, apart from general broadcast burning, for the specific purpose of destroying undesirable white fir and hemlock on Forest Service sales, was in 1910, on the Kaniksu National Forest. Here the hemlock and white fir were girdled in three seed plots, a belt of bark 2 to 3 feet high around the base of the tree being stripped off. The work was not done carefully and many strips of inner bark were left intact, connecting the root and the top. Consequently very few of the trees were killed and the wounded portion is gradually healing by the growth of new bark.

The objects of the girdling—to prevent seed production on the adjoining clean-cut area, and to lessen the shade in the seed plot—were both defeated, as the girdled trees produced heavy seed crops three years later, and the shade in the plots was not materially decreased.

The next work of the kind was on several cuttings in the white-pine type on the Coeur d'Alene National Forest, which was followed the next year by additional girdling on later sales. The method has since been specified for use in all white pine sales on the Coeur d'Alene Forest.

Two different forms of girdling were employed in these early trials. One was to strip off the bark around the base of the tree for a width of 2 to 3 feet, a method similar to that used on the Kaniksu National Forest. The other was to cut a notch 1 to 2 inches deep completely around the tree, with or without some stripping of the bark above or below the notch.

The stripping method was tried in the winter when the bark adhered so tightly that it would not peel but had to be cut off. The leaving of narrow strips of inner bark connecting the upper and lower ends of the wounds was, in many cases, unavoidable. The following year it was noticed that many of the hemlock and white fir trees which had been girdled by the notch or stripping methods still had green healthy tops and were bearing unusually heavy crops of cones. In order to determine whether the girdling had affected the fertility of these cones, two large hemlocks, one girdled by stripping and the other by notching, were cut, and a bushel of cones collected from each. Seed was extracted at the Northern Rocky Mountain Forest Experiment Station at room temperatures, never higher than 80° or 90° F., and duplicate tests of 500 seed each were made in sand flats in the experiment station greenhouse. The results are given in Table I.

TABLE I.—Germination of duplicate samples of 500 seeds each from girdled hemlock trees

Progress of germination	Number of days	Percentage of germination of seeds from—					
		Notch-girdled trees			Peeled trees		
		Test A	Test B	Average	Test A	Test B	Average
Beginning.....	19	11.2	5.0	8.1	0.8	0.2	0.5
Height.....	28	74.8	51.8	63.3	31.4	16.2	23.8
Last active.....	38	87.0	68.0	77.5	51.4	44.6	48.0
End.....	50	89.2	73.8	81.5	53.4	46.0	49.7

It is apparent from these tests that winter girdling, according to either the stripping or notching method, does not prevent the production of a crop of fertile hemlock seed the fall after girdling. Whether the girdling actually causes an increase in the amount of seed produced by these trees above the amount they would have borne if they had not been girdled is not definitely indicated. The crops from girdled trees appeared to be uniformly very heavy, but this occurred in an exceptionally good seed year for all species in the white pine region.

That the percentage of fertility of the seed from the tree girdled by stripping was but little more than half that shown by the tree girdled by notching, does not lead to any definite conclusion, for individual trees are known to vary widely in the quality of seed produced, regardless of external influences. The seed produced by the tree that had been stripped was sufficiently fertile (50 per cent) to restock the area effectively, so it is not possible to consider stripping the bark a better method than notching on the basis of this data. The other object of the girdling, the reduction of shade, was also defeated by the fact that the trees remained green all summer, at the time when white pine and larch seedlings were germinating and establishing themselves.

Thus during the first year after cutting, at the time when surface conditions for germination of desirable intolerant species are most favorable, the girdled hemlocks not only produced heavy crops of fertile seed, which on one particular area resulted in a stand of some 90,000 hemlock seedlings per acre by actual count, but also hindered by their shade the germination and establishment of intolerant white pine and larch on a considerable portion of the area.

Particular note was taken of the effect during the second season after these girdling experiments. A great majority of the completely girdled trees showed a very unthrifty appearance, and this was true, apparently to an equal degree, of the trees girdled by either the notch or stripping method. The girdling on the stripped trees was, however, more often incomplete on account of the strips of inner bark left, and consequently the notch girdling was effective in a larger percentage of cases of the trees girdled.

On several of the sale areas where the girdling had been done, the brush had been burned in piles that autumn. It was noticed that this brush burning had been very effective in killing both large trees

of the undesirable species, and thickets of saplings and advanced growth. The effect of this burning had been immediate, causing the crowns to die and the leaves to fall off within a short time after the burning. The effect of heat and fire on saplings and reproduction was surprisingly great, whole thickets being completely killed by small fires which actually came in contact with only a small proportion of the trees. On the other hand, the trees which had been girdled but not burned were just beginning to die and lose their leaves at the end of the second growing season after girdling, and after they had produced one good seed crop. The much greater effectiveness and value of the brush burning, compared to girdling, were very apparent. From this it is thought that girdling had best be done after brush has been burned, in order to supplement the burning and reduce the cost of cleaning up the area.

For the purpose of finding a more effective way of killing the white fir and hemlock by girdling or other means, an experiment was later installed which was planned to take into account available information on the physiological functions of trees.

In the light of present knowledge of these functions it is easy to account for the fact that the two girdled hemlock trees from which seed was collected remained green throughout the summer following the girdling and produced heavy crops of seed. The girdling did not prevent the movement of water, soluble salts, and stored food from the roots through the sapwood to the leaves. Nourished thus with this water and stored food, it was possible for the foliage to continue photosynthetic manufacture of new food during the summer after girdling. Since most or all of the surplus food could not return to the roots for storage, because of the girdling of the inner bark, it remained in the trunk and crown and was doubtless available for the production of a heavy crop of seed.

There is reason to think, furthermore, that girdling does not entirely prevent the movement of surplus food from the foliage to the roots. This theory is advanced by S. B. Elliott,² who cites instances of white pines that have lived from 5 to 22 years after girdling.

EXPERIMENTS IN GIRDLING AND POISONING

As winter girdling had not proved entirely successful, it was decided to try girdling at different times during the growing season, when, in accordance with the theory of food movement, there would be a minimum of stored food in the sapwood available for the production of seed and continued growth. Accordingly, it was decided to test 10 trees each of white fir and hemlock on each of the following dates:

May 15 (beginning of active growth).

June 15 (height of active growth).

July 15 (slackening of growth).

August 15 (height of storing of surplus food).

September 15 (slackening of vegetative activity at end of growing season).

² ELLIOTT, S. B. THE PROBLEM OF FOOD MOVEMENT IN TREES. *Forestry Quart.* 12: 559-561. 1914.

One hundred trees, varying in size from 7 to 28 inches in diameter, and in crown class from intermediate to dominant, were selected in an overmature white pine stand on the Priest River experimental forest. The average diameter of the 50 hemlocks was 15 inches at breast height, while that of the 50 white firs was 13 inches. Heights varied from 70 to 115 feet, averaging 90 feet for both species. The range in age was from 100 to 200 years or more.

Four different methods of straight girdling were used on each date, as follows:

- (1) Bark stripped off at base for a height of from 2 to 3 feet.
- (2) Trunk girdled with an axe notch cut in the sapwood to a depth of from 1 to 2 inches.
- (3) Bark stripped off at base for a distance of from 2 to 3 feet and an axe notch cut into the sapwood to a depth of from 1 to 2 inches, at about the middle of the stripped belt.
- (4) Notch from 2 to 3 inches deep, cut through sapwood with a saw and chipped out with axe.

In addition to the girdling, poisoning experiments were begun on a small scale. The following three methods of introducing the poisonous material were used:

- (1) Two short notches, 6 to 7 inches long and 2 to 3 inches deep, with their inner edges an inch or two lower than their outer edges were chopped into the sapwood on opposite sides of the tree. They were then filled with a saturated solution of either copper sulphate, copper acetate, or zinc chloride.
- (2) Four auger holes, 2 inches in diameter and 3 inches deep, were bored at the base of the tree on opposite sides, and chemicals were put in the holes, as saturated solutions (in the May treatments) or in dry form with water added (as in the June and July treatments). The chemicals used in this treatment were copper sulphate, copper acetate, zinc chloride, copper carbonate, lye, and sodium chloride.
- (3) A notch 1 to 2 inches deep was cut completely around the tree, as in method 2 for girdling only, and two 2-inch auger holes were bored at the base of the tree on opposite sides. These holes were filled with the same chemicals used in the foregoing test (2), a saturated solution being used in the May treatments and dry chemicals³ with water added in June and July.

EFFECT ON FOLIAGE

Table II gives a summary of the effect of different treatments on the appearance of the foliage, in every instance where such an effect was noticeable at the end of the season.

³ The reason for trying the dry chemicals was to see if the gradual dissolving and absorption into the sap would be any more effective than the rapid absorption of solution.

TABLE II.—*Effect of girdling and poisoning treatments during period of growth, as observed at end of season*

Treatment	Species	Date treated	Effect on tree		
			Un-thrifty ^a	One-fourth dead ^b	Dead
GIRDLING					
Bark stripped off at base for distance of 24 to 40 inches.	Hemlock	May 15	1		
	do	June 15	1		
	White fir	do		1	
	do	Aug. 15		1	
Girdled with axe notch 1 to 2 inches deep	Hemlock	Sept. 15	1		
	do	May 15	1		
	do	June 15	1		
	White fir	do			1
Barking and girdling combined	do	do		1	
	do	Aug. 15		1	
	do	do	1		
Notch sawed and chipped 1 to 2 inches deep	Hemlock	June 15			1
POISONING					
Notches 6 to 7 inches long filled with saturated solution copper sulphate.	do	May 15	1		
Four 3-inch auger holes filled with saturated solution copper sulphate.	do	do	1		
Notches filled with saturated solution copper acetate.	do	do	1		
Girdling notch and two auger holes filled with dry lye.	White fir	July 17			1
No treatment control	Hemlock		1		
T tal	Hemlock		9		1
	White fir		1	4	2

^a Distinct yellow-green color of foliage.
^b One-fourth of inner leaves dead.
^c Almost dead or dead after Sept. 15.

The damaging influence of the treatments at the end of the first season was, on the whole, negligible. Of 85 trees treated (omitting 8 untreated control trees included in the series, and 7 trees blown down early in the season), only 16 showed any apparent effect whatever. Of these, only 3 had died (1 hemlock and 2 white firs). The other 13 trees (8 hemlocks and 5 white firs) appeared to be distinctly more yellow-green in color than their untreated neighbors, and showed more than the ordinary number of dead leaves. Not much importance can be attached, however, to this yellow-green appearance, as it may easily have been due to slight differences in exposure, soil, natural vigor of the tree, or other factors. The dying of inner leaves may have been just a natural shedding, as indicated by the fact that among the unthrifty trees was an untreated control tree. It is interesting to note, however, that the three trees which died were all girdled with a notch, two of them being axe notches and one a sawed notch. The percentage of the total basal area of the tree occupied by the notch ranged from 13.5 to 65.3, averaging 39.8 per cent for hemlock and 44 per cent for white fir. There seemed to be no relation between the death of the three dead trees and this percentage, the notches on the two white firs being below average and that on the hemlock somewhat above.

Chemicals had been used in only four cases out of the 16. In two cases hemlock trees which showed a yellow-green cast to the foliage had been treated in open-chopped notches—in one instance with a

saturated solution of copper sulphate, and in the other with a saturated solution of copper acetate. In another case a white fir which had been both completely girdled with an axe notch and treated with dry lye placed in two auger holes, died toward the end of the season. The fourth case, a hemlock, showed signs of unthriftiness after a saturated solution of copper sulphate had been placed in four 3-inch auger holes bored in the trunk.

Since the copper sulphate, copper acetate, and lye showed no apparent effect in the several other cases in which they were used, it is probable that they had little or no influence in these treatments. It was noted in the treatments where dry chemicals were used that the more easily soluble materials—zinc chloride, lye, and sodium chloride—had been entirely absorbed, while the copper sulphate, acetate, and carbonate, were only partially dissolved, the residue amounting to from one-fourth to one-half or more of the amount of chemicals used.

One indication of the effect of girdling, it was thought, would be the extent to which the sapwood had dried out, since this would show the degree of restriction of the circulation of water and stored food upward from the roots to the crown. Accordingly, the depth of drying at the last observation, on November 17, was determined for a majority of the girdled trees. Measurements were taken at the center of the stripped portions, and inward from the inner edge of the notches.

Considerable irregularity was noticed in different trees and at different points on the same tree. The dryness on the stripped trees extended from 0.1 to 1.1 inch with an average of 0.4 inch. In the case of the notched trees, the drying extended 0.75 inch to 2 inches in from the innermost point of notch, with an average of 1.1 inch. The depth of the notches ranged from 1 to 3.75 inches, averaging 1.7 inches. The average depth of drying on the notched trees was 2.8 inches compared to 0.4 inch on the stripped trees.

Taking 15 inches as the average diameter at the point of girdling, the average reduction in the amount of wood available for the transmission of water and sap was 60 per cent of the cross-sectional area of the trunk on the notched trees compared to 5 per cent on the stripped trees. This difference indicates that the notching causes a twelve times greater restriction in the passage of water and food than does the stripping, and probably accounts for the fact that the only trees which died were those which had been notched.

Careful observations were made in the fall with field glasses, but no new cones were seen on any of the trees, although several cones left from the preceding year's crop were noticed on some of the hemlocks. This failure of the seed crop can not be attributed to the treatment, however, since there was an almost complete failure of the seed crop of all species in the region that year, due, probably, to severe frosts late in June.

TIME REQUIRED FOR TREATMENTS

The time required for each treatment was recorded. Table III gives the average figures obtained:

TABLE III.—Time consumed in girdling experiments

Kind of treatment	Western hemlock		White fir		Both species	
	Average d. b. h. ^a	Time per tree (1 man)	Average d. b. h.	Time per tree (1 man)	Average d. b. h.	Time per tree (1 man)
	Inches	Minutes	Inches	Minutes	Inches	Minutes
Bark stripped off.....	18	8	13	7	15	7
Girdled with ax notch.....	15	7	13	4	14	5
Stripped and girdled with notch.....	14	9	12	11	13	10
Girdled with sawed notch.....	14	11	11	11	12	11
2 to 4 chopped notches 7 inches long filled with chemical.....	12	8	13	5	12	6
4 auger holes 3 inches deep filled with chemical.....	14	10	13	9	13	9
Girdled with ax notch, 2 auger holes filled with chemical.....	16	10	12	8	14	9

^a Diameter at breast height.

It is apparent from this record that the method of girdling with and ax notch was distinctly faster than any of the other methods, requiring an average of 5 minutes of one man's time to a tree compared to 7 minutes for the stripping method, 10 minutes for the combined stripping and notch methods, and 11 minutes for the sawed-notch method.

CONCLUSIONS

No definite conclusions can be drawn from this work as yet because of the small number of instances showing effects of these treatments, and also owing to the possibility of developing further and more satisfactory methods of eliminating undesirable hemlock and fir. It seems probable, however, that girdling with an ax notch is the most desirable method, both in respect to cost and effectiveness. Also it seems that girdling in the spring and early summer (April, May, and June) is more effective than girdling during the late summer or winter.

So far, burning as a means of getting rid of these trees has not been thoroughly investigated, and it is planned to carry out further work along this line in order to determine just how far elimination can be accomplished when the slash from timber sales is burned.

COMPARATIVE SUSCEPTIBILITY OF ONION VARIETIES AND OF SPECIES OF ALLIUM TO UROCYSTIS CEPULAE¹

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INTRODUCTION

Most pathogenic fungi show a distinct variation in their ability to infect different varieties of their host plant. Such a difference in varietal susceptibility is usually the starting point for the breeding or selection of resistant varieties, which is the most effectual method for the control of plant diseases. With respect to onion smut, however, it has never been shown that there is any difference in the susceptibility of the numerous varieties under cultivation; in fact, no very comprehensive variety tests with this objective in view have been conducted. Walker and Jones (12)² tested a few varieties, and Whitehead (13, p. 449) in England tested 21 varieties of onions and 11 varieties of leeks (*Allium porrum*), but none showed resistance. Nevertheless, since there were numerous other varieties which had not been tested, so far as was known to the writer, it appeared worth while to collect seed of as many varieties as could be obtained and test them comparatively in the hope that some of them might show a degree of resistance which should warrant crossing and selection work.

The genus *Allium*, to which the cultivated onion belongs, is a large genus of some 250 species which are widely distributed over the earth. *Urocystis cepulae* was first reported on *Allium cepa* (4, 8) in 1857, in eastern Massachusetts. As early as 1881 (7) it was also reported on *A. porrum* (leek), in France. Cotton (3) reported it as more serious on this species than on onion in England. Clinton (2, p. 451) reported it as occurring on *A. nevadense* in the far West of the United States. Quite recently Zillig (14, p. 57) has also reported infection of *A. fistulosum* L., *A. globosum* Red., and *A. odorum* L. Thus this fungus has been found on five species of the genus besides *A. cepa*.³

A more thorough knowledge of the extent of its host range is desirable for at least three reasons:

(1) It might give us a clue to the origin of onion smut. This disease seems to have been noticed for the first time on onions in New England about the middle of the last century. Where it came from and where it had passed the preceding centuries, nobody seems to know. There is the strong probability that it came from some native American susceptible *Allium*; yet, with the exception of

¹ Received for publication October 22, 1924; issued, September, 1925.

² Reference is made by number (italic) to "Literature cited," p. 286.

³ After this paper had been forwarded by the writer for publication, a letter was received from Doctor Zillig stating that he had also been able to infect *A. cyaneum* Reg., *A. flavum* L., *A. huteri* Sund., *A. hymenorrhizum* Ledeb., and *A. obliquum* L.

Allium nevadense in the far West, no American *Allium* is known to be susceptible, so far as the writer is aware. If it were found that some one of our New England *Alliums* is subject to this disease, then an explanation of its origin would be easy.

(2) It would help settle the question of a possible relationship between *Urocystis cepulae* and some of the other species of *Urocystis* which occur on other species of *Allium*.

(3) In a genus as large as *Allium*, it is probable that there are some resistant species which may be crossed with *A. cepa* and that from the hybrid progeny of which a desirable smut-resistant onion might be developed. The initial step is to determine how resistant the different species are, if they are resistant at all.

EXPERIMENTS WITH ONION VARIETIES

From the catalogues of American seed houses, 25 onion varieties which seemed to be distinct were selected. Seed of 29 European varieties were obtained from H. Zillig, of the Biologische Reichsanstalt für Land-und Forstwirtschaft, at Trier, Germany, who also was investigating the same phase of onion smut and who kindly sent seed of each of the varieties which he was trying. Three hundred seeds of each of the 54 varieties thus obtained were planted April 25, 1924, in soil which was known to be very heavily infested with smut. When the seedlings came up there was seen to be considerable variation in the percentage of germination. Smut had appeared on all of the varieties after three weeks. All plants, irrespective of infection, were counted on May 30 to determine the percentage of germination, and at that time smut infection was estimated at about 75 per cent on all varieties and none offered evidence of being strikingly more resistant than others. The smutted ones had already begun to die and continued to drop off throughout the summer. On August 11, all the remaining plants were pulled and counted, and the percentage of infection was determined by comparing the number of healthy plants at that time with the number which had germinated on May 30.

The results are presented in Table I. They do not indicate that there is any important degree of resistance in any of the 54 varieties tried. Neither does the theory of some onion growers, that the red varieties are more resistant, find any support in these results. The pigmentation factor shows no relation to the resistance factor. There are, however, still other varieties which have not been tested, and it is possible that some more resistant variety may be found among them. It is therefore important that this line of work should be continued.

TABLE I.—*Susceptibility test of varieties of Allium cepa*

Name of variety	Source of seed	Number of plants May 30	Healthy plants Aug. 11	Percent-age of infection
American varieties:				
Australian Brown.....	Livingston Seed Co.....	195	58	70
Bermuda Yellow.....	F. H. Woodruff & Sons.....	210	33	84
Crystal white wax.....	do.....	177	21	88
Early Barletta.....	do.....	163	39	76
Early Neapolitan Marzajola.....	D. M. Ferry & Co.....	0	0	-----
Early Yellow Cracker.....	Fottler-Fiske-Rawson Co.....	73	2	98
Extra Early Red Flat.....	F. H. Woodworth & Sons.....	176	17	90
Extra Early Yellow.....	do.....	152	12	92
Giant White Italian Tripoli.....	D. M. Ferry & Co.....	0	0	-----
Isbell's Early White Sugar Ball.....	S. M. Isbell & Co.....	52	0	100
Isbell's Evergood Red Globe.....	do.....	109	10	90
Large Red Wethersfield.....	F. H. Woodworth & Sons.....	95	10	89
Mammoth Silver King.....	Livingston Seed Co.....	78	12	84
Ohio Yellow Globe.....	F. H. Woodworth & Sons.....	66	5	93
Prizetaker.....	do.....	136	28	78
Red Italian Tripoli.....	Fottler-Fiske-Rawson Co.....	17	3	80
Southport Red Globe.....	F. H. Woodworth & Sons.....	83	15	81
Southport White Globe.....	do.....	132	14	89
Southport Yellow Globe.....	do.....	182	21	88
White Adriatic Barletta.....	Fottler-Fiske-Rawson Co.....	56	1	98
White Bunch.....	Alexander Forbes & Co.....	125	26	79
White Portugal.....	F. H. Woodworth & Sons.....	94	5	94
Yellow Danvers Flat.....	do.....	150	20	86
Yellow Dutch or Strassburg.....	do.....	136	12	91
Yellow Globe Danvers.....	do.....	166	9	95
European varieties:				
Blassrote hollandische.....	H. Zillig.....	119	10	91
Blut rote Ulmer.....	do.....	147	36	75
Bornaer Riesen.....	do.....	64	10	85
Bronzekugel.....	do.....	7	0	100
Braunschweiger schwarzrote.....	do.....	15	2	86
Calbenser Lokalsorte.....	do.....	71	22	68
Eisenkopf.....	do.....	103	17	83
Erfurter blassrote.....	do.....	26	4	84
Franzoesische oder spanische weisse.....	do.....	155	29	81
Gelbe Birn.....	do.....	85	18	78
Hollandische blutrote plattrunde harte.....	do.....	119	16	87
Hollandische gelbe plattrunde harte.....	do.....	96	10	89
Hollandische silberweisse plattrunde harte.....	do.....	90	6	93
Liegnitzer Lokal-Sorte.....	do.....	145	16	88
Runde gelbe Stuttgarter Riesen.....	do.....	38	6	84
Silberweisse Konigin.....	do.....	116	12	89
Silberweisse plattrunde fruhe.....	do.....	127	11	91
Strohgelbe (hellgelbe) plattrunde.....	do.....	33	1	97
Tripoli Rocca.....	do.....	118	17	85
Vertus.....	do.....	58	8	86
Weisse fruhest Fruhlings-Zwiebel.....	do.....	163	10	93
Zittauer Riesen—				
Blutrote runde.....	do.....	18	0	100
Gelbe runde.....	do.....	7	1	85
Schwefelgelbe.....	do.....	112	19	83
Silberweisse runde.....	do.....	161	20	87
Blassrote plattrunde harte Erfurter.....	do.....	162	4	97
Dunkelrote plattrunde harte Braun-schweiger.....	do.....	191	8	96
Grosse gelbe runde Zittauer Riesen.....	do.....	125	2	98
Hollandische gelbe.....	do.....	110	0	100

EXPERIMENTS WITH OTHER SPECIES OF ALLIUM

During the last three years the writer has been attempting to determine the susceptibility of all species of *Allium* of which viable seed could be obtained. The seeds were planted in soil known to be heavily infested with the smut fungus and when the seedlings came up the percentage and severity of infection were recorded weekly until the plants were well grown and there seemed no more probability of new infections. Progress of the investigation has been slow on account of the difficulty of securing viable seed. Letters sent to the American seed houses and botanical gardens where one might expect

to find seed of wild species netted two or three species which did not germinate. Somewhat better success was had in Europe and most of the seed which the writer has obtained came from Kew Gardens, Haage & Schmidt, of Erfurt, Germany, H. Zillig, of Trier, Germany, and Vilmorin-Andrieux & Co., at Paris. Only last summer did the writer succeed in getting seed (or bulblets) of all the three native New England species of *Allium*. *Allium* seed seems to lose its vitality very rapidly, and seed more than a year or two old frequently can not be made to germinate. The writer thinks it is possible, of course, that he has not been able always to provide in the plots the special conditions which may be necessary to bring some species to germination. Altogether seed of about 100 species has been tried and the seed of only 39 have germinated.

In the course of this work it has been observed that there are gradations in the severity of infection. On this basis all the species may be divided into the following classes:

Class 1. Most susceptible class. Sori first appear in the cotyledons and many of the plants die at this time. Those which survive may slough off the disease, or lesions may appear in the successive true leaves, ending in the final death of the plant before it forms seeds.

Class 2. Less susceptible than class 1. Smut sori occur only in the cotyledons, and many plants die in this stage, but if they survive no lesions appear in the true leaves.

Class 3. Species barely susceptible in that smut sori may be found in the cotyledons, but the plants are not killed in this or later stages and no sori appear in the true leaves.

Class 4: Entirely resistant species in which smut lesions have not been observed in any of the parts.

In infested soil from the same field three different plantings were made. Nineteen species were planted May 1, 1923 in flats kept out of doors; 40 species in a greenhouse bench on October 11, 1923; and 64 species in an outdoor bed on April 25, 1924. Some species were tested in all three plantings, some in two, and some in only one, depending on the supply of seed and the need of further testing. In cases where fewer than 100 plants came up this fact is mentioned in the following notes on the individual species.

CLASS 1

Allium Cepa L., to which all our varieties of cultivated onions belong, is typical of Class 1. All infection seems to take place through the cotyledon. A large percentage of infected plants die in this stage. If no sori occur in the cotyledon, none will be found in the true leaves which follow. Sometimes, even when the cotyledon is affected, the fungus fails to gain entrance into the growing point and no lesions will be found in the following leaves. In the latter case, the bulb and seed are developed normally. More often, however, the parasite gets into the growing point and each successive leaf will have spore sori, in which case the leaves are short, distorted, and brittle, and the dwarfed plants die in various stages of development throughout the summer. Some of them form bulbs as large as an inch in diameter with longitudinal black spore pustules in the outer scales, but they usually rot before they are harvested. It is doubtful whether a plant which has spores in the first leaf ever recovers. In the soil

which was used for the comparative susceptibility experiments, 80 to 100 per cent of the seedlings which appeared died before harvest.

Allium fistulosum L. (Welsh onion). This onion reacts in every way like *A. cepa*, except that the percentage of infection is not so high. In the first planting 50 per cent were infected, 5 per cent in the second, and 25 per cent in the third. Infected plants succumbed in various stages of development. This species is a robust, rapid grower, and lives through the winter out of doors. There was no difficulty in getting a high percentage of the seed to germinate. Zillig also found this species susceptible.

Allium ascalonicum L. (Shallot). Only one planting was made, and about 100 strong plants started growth. Ninety per cent had smut in the cotyledons, and those which did not die in this stage had smut in the successive leaves and died throughout the summer, just as did *A. cepa*.

Allium hookeri Thwaites. In the first planting 10 per cent were infected. In the second planting 95 per cent were infected, and the majority of the plants died at an early stage. Sori were found in the fourth and fifth leaves, but no smutted plant reached maturity. This species seemed just as susceptible as *A. cepa*.

Allium libani Boiss. In the first planting 50 per cent had smut, and in the second 95 per cent of the cotyledons had it. A large percentage of the plants died in that stage. Only a few showed smut in the later stages, and none of the diseased ones developed to maturity.

Allium senescens var. *giganteum*. About 100 plants started growth from the one sowing made. Smut lesions were in the cotyledons of almost all of them, and many died in this stage. Lesions also occurred in the early true leaves. By the middle of the summer only 15 plants had survived, but they were free from smut. This appears to be a very susceptible variety and is remarkably different in this respect from the rest of the species (if, indeed, this is only a variety of the form listed below as *A. senescens*, the seed of which was received under the name of *A. fallax*).

Allium huteri Sund. Although a large number of plants came up, they all died before the first leaf was fully developed, and all were found to be full of smut lesions. This species appears to be so susceptible that it never gets much past the cotyledon stage in an infested soil.

Allium flavum L. There was 95 per cent infection in the cotyledons of the hundreds of plants of this species, and a heavy mortality at this stage. Sori were found in young leaves of some which continued to live, but no smutted plants were found later in the summer. The mortality seems to be all in the young stages.

Allium schoenoprasum L. (chives), is much more resistant than *A. cepa*. Infection occurred in only about 2 to 5 per cent of the cotyledons. Later in the summer sori were found in the leaves. Affected plants did not divide and produce tufts of shoots as did the healthy ones. All infected ones died before the end of the summer.

Allium schoenoprasum var. *sibiricum* L. Only two plants came up when this seed was planted. One of the two had smut sori on it.

Allium nutans L. This is a very susceptible species. Two plantings were made, and some hundreds of seedlings came up. Ninety

per cent of these seedlings had smut in the cotyledon, and most of them died in this stage, but a few continued to develop and had smut sori in the true leaves up to the fourth or fifth leaf, but no sori were found on any plant that was more mature. This species almost falls in class 2.

Allium neapolitanum Cyr. Not more than 1 per cent of the hundreds of seedlings which grew from one planting showed smut. Lesions were found, however, in the first true leaf. Hence this species is placed in class 1, although it appears to be very resistant.

CLASS 2

Allium porrum L. (leek). In England this species is said to be attacked more severely than *A. cepa* (3, p. 170). Zillig also was able to infect it, but neither he nor Cotton described the effect of the disease on the plants. Malbranche (?) stated in 1881 that 20 per cent of the *porrettes* (young leeks) in certain gardens in Rouen were attacked, and that the disease had established itself in the blades and especially the bases of the leaves. The writer made two plantings and had no difficulty in raising hundreds of plants to maturity. The first planting showed 15 per cent of infection in the cotyledons, and the second 10 per cent, but only a very few of the plants died. The writer has searched in vain for sori in the true leaves of the plants. The damage caused by the parasite here is negligible. These observations are surely at variance with those of Malbranche and other European investigators. Apparently the disease is more serious on leeks in Europe than in America.

Allium angulosum L. From the one planting made several hundred seedlings were obtained. Ninety-five per cent of the cotyledons had smut, and more than half of the plants died in this stage. Those which survived, however, showed no trace of smut in the leaves. This seems to be an extremely susceptible species, but the mortality is all in the early cotyledon stage.

Allium nigrum L. Considerable interest is attached to this species because of its relation to *A. magicum*, on which Passerini collected a smut which he named *Urocystis magica*. (In the past there has been considerable discussion as to whether *U. magica* is identical with *U. cepulae*. If it is, the onion smut fungus may well be of European origin. *A. magicum* is either a variety or a synonym of *A. nigrum*, according to the authority one wishes to follow.) One sowing was made, but the germination was poor; only about 30 seedlings grew. One-third of these had smut in the cotyledon, but none was observed in the later leaves, although all the plants were grown to full maturity and produced blossoms and seeds. The original specimens of *U. magica*, collected by Passerini and examined by the writer in the Harvard herbarium, were on very large leaves. The fact that the large leaves of *A. nigrum* have not been observed to become infected would indicate that the fungus Passerini collected and named was not identical with *U. cepulae*. More thorough infection tests should be made, however, the writer believes.

Allium pulchellum G. Don. Three plants came up and died of smut in the cotyledon stage.

Allium sikkimense Baker. Only two plants came up. One showed smut lesions and soon died.

CLASS 3

Allium volhynicum Bess. Two plantings were made. Ten per cent of those of the first planting had smut in the cotyledons, and 50 per cent of the second planting had it. No lesions were observed in the true leaves.

Allium polyphyllum Kar. and Kir. Two plantings were made, but the germination was poor. Only six plants were obtained. Smut lesions occurred in the cotyledons of half of them, and some died later, but the writer is not sure that the smut killed them. Further tests might place this species in class 1 or 2.

Allium scorodoprasum L. Same general condition as for the preceding. Only a few plants came up, but some of them had lesions in the cotyledons.

Allium obliquum L. Of several hundred plants which came up 90 per cent had infected cotyledons, but the later leaves showed no sori.

Allium senescens L. (*A. fallax* Schult). Out of about 150 seedlings from the first planting, smut lesions were found in only five cotyledons. In a second planting, 2 per cent of the cotyledons had smut. No lesions were observed in any of the leaves, and it was not observed that any of those which had smut in the cotyledons died. Apparently here is a very resistant species, but not immune.

Allium ampeloprasum L. Only a few seedlings were obtained. About half of them had sori in the cotyledons.

Allium darwasicum Reg. Only 14 seedlings resulted from the one planting made. Three or four of them had smut lesions in the cotyledons.

Allium montanum Sib. and Sm. Twenty seedlings grew from the one planting. Two of them had infection in the cotyledons, but no infected ones were observed later.

Allium paradoxum Don. Of the three plants which came up, one had cotyledons lesions, but no disease was observed in later stages.

Allium odorum L. Three plantings with seed from as many different sources were made, and hundreds of plants were grown. No smut was observed in any of them. The results were at variance with those reported by Zillig, who was able to infect this species. Subsequent to the third planting, Zillig kindly sent some seed which he obtained from the Botanic Gardens in Munchen. When plants grew from these, about 10 per cent had lesions in the cotyledons, this suggesting the possibility that in this species we may have varieties or strains which differ in their susceptibility to the smut organism.

Allium recurvatum Rydb. Of the 50 plants which came up, one-half had smut in the cotyledons, but no lesions were observed later on the true leaves. Although many of these plants died in a young stage, it was not evident that the smut was the cause.

CLASS 4

Allium oreoprasum Shr. Two plantings were made, and hundreds of seedlings grew, but no smut sori appeared on any of the parts at any time. As far as these trials showed, this species is perfectly immune.

Allium moly L. Only one planting with this species was made. The germination was poor, and only a dozen plants were obtained;

none of them showed infection in any stage. Although the writer has had these plants growing for two years, none of them has produced seed.

Allium heldreichii Boiss. Only three plants came up, and none of them was infected.

Allium macranthum Baker. About 50 plants were obtained from the single planting. No lesion was found at any time on any of them.

Allium subhirsutum L. About 100 plants came up. No smut was observed in them at any stage.

Allium roseum L. A very few plants came up and soon died, but no smut was observed. The writer is of the opinion that further tests should be made with this species.

EXPERIMENTS WITH SPECIES WHICH REPRODUCE BY BULBLETS

It has long been known that when onions are started from sets (small bulbs) they are entirely free from smut. In a previous publication, the writer has shown that all infection of onions takes place through the cotyledons (1). As there are no cotyledons when a plant starts from a bulblet, it is easy to understand why onions from sets are immune. There are many species of *Allium* which never produce seeds but reproduce by small bulblets which form in dense heads in a position much like the seed clusters of seed-bearing species. There are other species which produce both bulblets and seeds, and all gradations may be found between these two methods of reproduction. The most common native wild onion in New England (*Allium canadense*) reproduces by bulblets only. Although the writer has found plants which were producing flowers and seemed to be setting seed, he has never found that the seed matured in this section of the country. Bulblets of this species have been planted and many plants grown to maturity, but no smut has been found at any time. The results were the same using bulblets of *A. roseum*. The writer has not been able to obtain viable bulblets of other species, but there is every reason to believe that the results would not be different. Probably none of the bulblet-producing species are ever infected. In view of the widespread tendency toward the bulbiferous habit throughout the genus *Allium*, it is an interesting speculation as to the influence which the smut fungus may have had in eliminating the seminiferous strains and forcing by natural selection the development of bulbiferous species.

EXPERIMENTS WITH THE WINTERBECK ONION

Some seed labeled "Winterbeck Zwiebel" were received from Haag & Schmidt, Erfurt, Germany. The writer is not sure whether this should be considered a distinct species or only a variety of *Allium cepa*. It comes up more quickly than the common onion, and grows more rapidly, but does not develop a large bulb, and the bulb divides more readily, somewhat with the same general habit as the Welsh onion and the multiplier (or Egyptian) onion. Unlike the latter, however, it does not produce a head of bulblets but reproduces entirely by seeds which form in heads, as in the case of the common onion. It is a stronger grower and more hardy than the common onion, less susceptible to damping-off and thrips. It is

regarded as all but immune to smut. Not more than 2 or 3 per cent showed smut in the cotyledons, and no smut was seen in any later stage. In fact, smut is an entirely negligible factor. This seems to be the logical parent species for the breeding of a smut-resistant onion by crossing.

THE RELATION OF UROCYSTIS CEPULÆ TO OTHER SPECIES OF UROCYSTIS ON ALLIUM

As far as the writer is aware, no *Urocystis* other than *Urocystis cepulae* has ever been found on the cultivated onion. Investigators who have found the disease agree as to the remarkable constancy of characters of this species so that not even a variety has been suggested. On other species of *Allium*, however, four other species of *Urocystis* have been found, and from the first description of the species up to the present mycologists and pathologists have discussed the relationship of these other species of *Urocystis* to *U. cepulae* without reaching any agreement. Some have considered all five forms as distinct species, some would unite them all under one species, i. e., regard the onion smut fungus as a variety of or identical with some of the others. Various other combinations are proposed. Not until all of these species have been cultured and studied and cross inoculations made on all host species concerned will this question be definitely settled; but it is hoped that the inoculation experiments just described and supplemented by examination of exsiccati of the pathogenes may contribute something to its solution. Let us examine briefly the other four species.

Urocystis magica Pass. was named by Passerini from specimens which he collected on *Allium magicum* at Parma, Italy, in 1875, and distributed in Rabenhorst's *Fungi europaei* as No. 2100 and in von Thuemen's *Mycotheca Universalis* as No. 223. The writer knows of no record that the fungus has ever been collected again on that host in Italy or elsewhere. All subsequent literature merely refers to the original description or consists of observations on studies of Passerini's original collection. It is remarkable that a fungus which was so abundant that it could be furnished in plentiful supply for distribution in two exsiccati should never have been collected again. The writer had occasion to study specimens of both of these exsiccati in the Harvard University herbarium. The long (some of them more than an inch) raised sori were on broad flat leaves. *A. magicum* is considered by most phanerogamic authorities as a synonym or a variety of *A. nigrum* L. This latter species, however, does not have flat leaves, but linear, terete leaves. Apparently, then, the plant on which Passerini collected the parasite was not *A. nigrum* but some other species. The spore balls contained a single spherical, or short, oval, brown central cell surrounded by hemispherical accessory cells. Only rarely did the writer find two fertile cells in a spore ball, and these were not attached by their surfaces, but were held together because surrounded by a common layer of sterile cells. In shape, color, and arrangement of all parts these spores could not be distinguished from those of *U. cepulae*. There is, however, a constant difference in size; the spores of *U. magica* were larger. Fifty spores from each species were measured under identically the same conditions. The central cell of *U. magica*, mounted in lactophenol, measured 14.27 by 15.55 μ as compared with 11.04 by 11.75 μ for *U.*

cepulae. The diameter of the entire spore ball of *U. magica* was 22.19 μ , while that of *U. cepulae* was 16.15 μ . All dimensions are somewhat greater when the spores are measured in a potassium hydroxide solution or when free. When the spores have been kept in a dry state for a long time, the accessory cells collapse but become distended again when treated with a weak solution of potassium hydroxide. Farlow (5, p. 114) considered *U. magica* as identical with *U. cepulae*. Schroeter and Winter (Die Pilze, p. 121, 1884) agree with Farlow and unite all the forms discussed here under *Urocystis colchici* Schlecht. Thaxter (11, p. 144) and Clinton (2, p. 451), on the other hand, point to the differences in the size of the spores as sufficient reason for considering the two species distinct. If by any chance the host plant of *U. magica* was really *A. nigrum*, the fact that the writer was unable to cause infection except on the tiny cotyledons, while in the exsiccati the fungus is on large leaves, seems to the writer another argument against the identity of the two. The constant difference in the size of the spores as observed by all investigators, and the difference in host plants, seem to be sufficient reasons for regarding *U. cepulae* as a species distinct from *U. magica*.

Another species of *Urocystis* has been reported on *Allium rotundum* in Europe by various investigators, and has usually been referred to *U. colchici* Schlecht., a common species there, occurring on a long list of Liliaceae. Some authors give to the form which occurs on *Allium* the rank of a variety, *forma Allii* under *U. colchici* because of certain morphological differences. The writer made a study of a specimen of *forma Allii* (Fückel Fungi rhenani No. 2217) collected by Fückel in Austria on *Allium rotundum*. All characters and dimensions were so nearly identical with those of *U. magica*, which were studied at the same time, that, from a morphological standpoint, no reason could be found for considering the two as distinct. These two forms are also doubtfully united by Liro (6) in his recent excellent monograph of the genus *Tubercinia*.

After the variety on *Allium rotundum* has been removed from *Urocystis colchici*, the species as it occurs on other hosts is easily distinguished from *U. cepulae* because the spore balls commonly are attached in glomerules of 3 to 5 or more, and a single spore ball may have 2 to 3 fertile cells. All dimensions are also larger than those of *U. cepulae*.

Rostrup (9, p. 153) found a species of *Urocystis* on *Allium ascalonicum* in Denmark, in 1890, and referred it to *U. cepulae*. Liro, however (6, p. 50), refers it to his newly described species *U. ferruginea* which differs sharply from *U. cepulae* in the red brown color of the spore powder, and in the angular spores attached in groups of six or more. The writer has not had an opportunity to examine this species, but, judging from the description given by Liro, there would seem to be no question as to its distinctness and no occasion for confusing it with *U. cepulae*.

In 1911, Schellenberg (10, p. 14) described the species *Urocystis allii* from a specimen collected in Switzerland in 1902 on *Allium subhirsutum* and distributed in von Thuemen's *Mycotheca universalis* as No. 1219. Although in morphological characters it seems to resemble *U. cepulae* even more closely than does *U. magica*, both Schellenberg and Liro consider it as distinct. As previously mentioned in this paper, *A. subhirsutum* L. was found by the writer to be entirely immune to *U. cepulae*, which is regarded as evidence con-

firming the distinctness of the two species of *Urocystis*. Another collection of a *Urocystis* on *A. oleraceum* L. was also referred to this same species by Schellenberg.

ORIGIN OF THE ONION SMUT

What bearing have the facts set forth in this paper on the problem of the origin of onion smut? The writer believes that there are good morphological and biological reasons for considering the smut fungus as a distinct species from any of the *Urocystes* found on European species of *Allium*. It should also be pointed out in this connection that even if it should be found that some one of the European species named in recent years was identical with *Urocystis cepulae*, this would not indicate a European origin for the disease. It seems to the writer that it would be much more logical in that case to conclude that the fungus was first on onion and passed from the onion over to the other species where it was collected. In fact, in view of the wide host range which the writer has demonstrated for the onion smut organism, it would seem remarkable if it should not be taken some time by some collector on another species. During the 50 years that it has been in Europe it has had abundant opportunity to spread to other hosts.

In brief, in the writer's opinion, there is not the least evidence that the disease is of Old World origin. On the contrary, there is excellent circumstantial evidence that it did not exist there on any species previous to its discovery in America. Onions have been grown and used by every civilized people in the Old World at least since the building of the pyramids. In all that time onions certainly must have come in contact with every disease which occurred on other species of *Allium* indigenous to the countries where they grew. Smut is by no means an inconspicuous disease, and if it had been present in the Old World it very likely would have been mentioned before 1872.

Some of the susceptible species mentioned in this paper are native to the New World, and probably further search will add to the list of our indigenous susceptible ones. Some one or more of these was probably the original host. Possibly it was a native of the far West in this country, like the susceptible *Allium nevadense*. For many years after the onion was brought to America smut seems to have been unknown. The writer offers the theory that as the frontier of civilization advanced into the West, taking along with it the onion, the onion came into contact with the organism in its native home, and that the spores could easily have been taken back to New England in the natural course of commerce, with the result that the disease first became prominent in a great intensive onion center, such as eastern Massachusetts was at that time, rather than in the isolated gardens of the pioneers. It may be significant that smut first appeared within the next decade after the California gold rush of 1849.

SUMMARY

None of the 54 varieties of cultivated onions tested showed any considerable resistance to smut.

Out of 39 species of *Allium* tested, 8 seemed to be immune, and 31 showed varying degrees of susceptibility.

Thirteen species seemed very susceptible, and are in the same class as the common onion.

Smut occurred in the cotyledons of 13 more species, but did not kill the plants or occur in the true leaves.

In five other species it occurred in the cotyledons and killed many of the seedlings in that stage, but did not appear in the true leaves.

Smut did not occur in species which reproduce by bulblets.

On this basis one might expect to find among the 250 known species of *Allium* at least 150 which are susceptible to smut.

Urocystis cepulae is considered by this writer as distinct from all other species of *Urocystis* which have been reported on other members of the genus *Allium*.

All the evidence the writer has indicates that this parasite lived originally on some wild American species and thence passed over to the cultivated onion.

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THE NITROGEN CONSTITUENTS OF CELERY PLANTS IN HEALTH AND DISEASE¹

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INTRODUCTION

Studies of the comparative chemical composition of plants in health and disease have, in the main, been few and the results obtained have not been such as to warrant generalizations as to the effects the various pathogenes bring about. Plant pathology has lagged conspicuously behind animal pathology in this regard, for in the latter science chemical pathology is a definite field with a large accumulation of data (27),² and these facts have permitted certain generalizations. In plant pathology the nature of the disturbance caused by the parasite can only be surmised, as the physiology of plants in disease is largely unknown and awaits a fuller knowledge of the physiology of plants in health.

In the early developments of agricultural chemistry, countless analyses catalogued the composition of plants. But these analyses, largely made to determine relative food value, do not permit the contrasting of plants in health and in disease. There have been observations made upon the development of certain chemical substances in the course of disease—pigments, toxic substances, enzymes, etc.—but these results are fragmentary and restricted in application.

That diseased plants are profoundly changed by the action of pathogenes is common knowledge, and the terms applied, decomposition or decay, indicate the general concept of the nature of the process which has taken place in disease production.

REVIEW OF LITERATURE

Müller-Thurgau (17, p. 61) has shown that the must from rotted grapes is poorer in nitrogen than that from sound grapes. The organisms producing the rot were found to use first the easily assimilated stuffs and accordingly the must from rotted berries was found to have nitrogen compounds present in far less extent and in a form less suitable for fungous growth than was the case with must from sound fruit. Similarly, he has also shown (16) that with rotted pome fruits, a clear sap almost free from nitrogen is obtained, in marked contrast to what is obtained from sound fruit.

Recently more comprehensive studies on the chemistry of healthy and diseased plants have been published. In a preliminary article, Boncquet (5), in discussing the chemistry of the mosaic disease in sugar beets, states, that dentrification sometimes takes place in

¹ Received for publication July 25, 1924; issued September, 1925. The chemical analyses presented in this paper were made by L. J. Klotz (14) who has developed the special methods for dealing with the *Septoria apii* material. The work with *Cercospora apii* was conducted in the laboratory of R. C. Huston at the Michigan Agricultural College. The writers wish to acknowledge indebtedness to him for advice. They are also indebted to C. S. Robinson for criticism of the manuscript.

² Reference is made by number (italic) to "Literature cited," p. 299.

diseased plant tissues whereby nitrates are converted into nitrite and ammonia. This result led this worker to attribute the disease in question and other diseases of similar nature to denitrifying bacteria.

We are indebted to True and his associates (23) for extensive and thorough-going analyses of plants in disease and health made in connection with their studies on spinach mosaic. In this investigation ash content, oxidase reactions, carbohydrate production, and nitrogen metabolism of healthy and blighted spinach were compared. The last named phase of the work was carried out by Jodidi, Kellogg, and True (23, p. 385-404), and since this part of the investigation was most extensive and the methods employed have since been the basis of other investigations, it will be considered in some detail. They found, as a result of their comparative analyses, that in diseased spinach there was a lower percentage of total nitrogen and of acid-amide nitrogen than in healthy spinach, and they explained this relation by the assumption that denitrification takes place in those tissues whereby a part of the nitrogen is lost either as elementary nitrogen or in the form of ammonia. They also found a higher proportion of ammoniacal nitrogen in the diseased material than in the corresponding healthy tissues, and the suggestion was made that the reason for this is also to be sought in the processes of denitrification, whereby a part of the nitrites is further reduced to ammonia.

In 1920, Jodidi, Moulton, and Markley (13) reported additional analyses along the same lines as those of the previous year, in order to corroborate the early results by observations on spinach taken from various fields and at other seasons. The results of this work seem to agree closely with the results of the earlier work. The presence of nitrites in the diseased plants postulated in the former article was definitely shown.

Jodidi (12) in 1920 gave the results of analyses of healthy and diseased cabbage. The diseased cabbage analyzed by him is described as showing a metallic color between the veins, only the spaces nearest to the veins being green. The leaves were brittle and their margins were frequently of a uniform yellow color. Coupled with these signs there was a poorly developed root system and a stunting. It is definitely stated that preliminary experiments to demonstrate the infectious nature of the disease had thus far led to negative results. The work previously mentioned had shown a certain type of the nitrogen distribution in mosaic spinach as contrasted with that in healthy spinach. Jodidi found that the diseased cabbage showed the same relations in the chemical constituents as compared with the healthy cabbage, and he concluded: "Judging from the striking similarity of the analytical evidence presented with regard to the various nitrogenous compounds occurring in healthy and blighted spinach plants, on the one hand, and in normal and diseased cabbage plants on the other, and taking into consideration that spinach blight belongs to the type of mosaic diseases, it appears logical to relate the cabbage disease caused by denitrification to the latter type."

Further contributions to the biochemistry of plants in health and disease have been made by Willaman and Sandstrom (28), but in this work the chief emphasis has been placed upon other constituents than the nitrogenous ones.

Reference also should be made to the contribution of Doby (8) who studied the biochemical relations of healthy potatoes and potatoes affected with leaf roll and who found lower insoluble protein content in diseased than in healthy tubers, the low protein content being the net result of higher oxidase content of the diseased tubers which lead to a great combustion of the cleavage products of the protein.

EXPERIMENTAL WORK

It was thought desirable to secure for comparison with the work on a mosaic disease which has been cited, analyses from fungous leaf blights where the effect of the parasite brought about a definite circumscribed killing effect upon the leaf tissue. Celery leaves affected with the disease known as "early blight" caused by the fungus *Cercospora apii* Fres. were first used for this purpose. The analyses along with a statement of the methods employed are given below.

For further evidence upon the nature of the changes in the nitrogenous constituents of plants as brought about by fungous attack, material from another celery disease, "leaf spot" caused by *Septoria apii* Chester, was taken. In this disease, the effect upon the tissues is more pronounced than in the "early blight," the fungus commonly producing in its last stages a complete rotting of the affected leaflets and stalks.

The materials chosen, therefore, represent two rather widely differing types of disease, and the analyses given reveal something of the type of decomposition produced by the parasites.

METHODS USED WITH CELERY BLIGHTED BY *CERCOSPORA APII* FRES

The methods used were in the main those employed by Jodidi and his collaborators (13). All the determinations, except water content, were made with diseased and healthy celery of one variety, Easy Bleaching, which was collected from plants of the same age growing in the same garden plot. The work was done entirely on mature leaflets. The lesions on the blighted leaves involved approximately from one-sixth to one-fourth of the leaf area. The leaflets were spread in thin layers on cheesecloth and dried at room temperature for three days and then placed for three days in an electric oven which was maintained at a temperature of 49° to 54° C. The dried materials were next rubbed through a 40-mesh sieve, then mixed to assure more uniform sampling and finally placed in jars and tightly sealed. The dried, powdered leaflets thus made ready for use were of about the fineness of table pepper. The color of the powdered blighted material was an ashen, grayish green, while that of the healthy material was distinctly chlorophyll green.

The total nitrogen was determined by the Kjeldahl and Kjeldahl-Gunning methods, 2 gm. samples of the dry, unpowdered leaves being used in the first method, 2 gm. samples of the more representative powdered materials being used in the second method. It may be said here that in all ammonia distillations 4 per cent boric acid solution was used as the receiving liquid and brom-phenol blue indicator in the titration (19).

Nitric nitrogen was estimated by two different methods, F. M. Scales' zinc-copper couple reduction method (9), and the Schulze-

Tiemann nitric oxide gas method. (For literature on this and other methods used by Jodidi *et al.* see literature references (12, 13, 23).) According to Jodidi's procedure, 12 gm. each of healthy and blighted celery were repeatedly extracted and thoroughly washed with 85 per cent alcohol. Milk of lime was then added to the combined extracts and washings, which were then evaporated to dryness in a vacuum oven at low temperature. The residue was taken up with hot water, lead acetate solution added, and the whole filtered, washed, and the combined filtrate and washings made up to 2 liters. In the Scales' method 250 c. c. samples of the extract were placed in 500 c. c. Kjeldahl flasks containing 80 gm. of the Zn-Cu couple coils. Five grams of c. p. sodium chloride and 1 gm. of c. p. magnesium oxide were added and the ammonia from the nitrates thus reduced distilled into 50 c. c. of 4 per cent boric acid. In the gas method 250 c. c. aliquot portions of the same extract were used and the work carried out exactly as described (1, p. 312-315).

Distillation with magnesia not giving dependable results in the estimation of ammonia nitrogen, a modification of Grafe's method of distillation *in vacuo* at low temperature was employed. Ten-gram samples were placed in 1-liter flasks, treated with 25 c. c. of concentrated sodium chloride solution, 35 c. c. of water, and 15 c. c. of alcohol. Then the apparatus was carefully made tight, 15 c. c. of saturated sodium carbonate added by means of the separatory funnel, and the water pump turned on. The temperature of the water bath was then raised to 25° to 29° C., at which temperature it was held for 3 hours and then raised to 40° to 43° C. The water pump reduced the pressure to 21 to 52 mm. mercury, the average being 28 mm.; 50 c. c. portion of a 4 per cent boric acid solution were used in the Peligot tubes to receive the ammonia. The Peligot tubes were placed in the same ice bath and attached by a Y to the same water pump. Near the end of the 6 to 7 hour distillation period 15 c. c. more of alcohol were cautiously added through the separatory funnels. The distillation was continued 20 to 30 minutes longer, during which time the particles of ammonia-containing moisture that collected in the necks of the flasks and the conducting tubes were removed by squirting a stream of boiling hot water against the exterior of the glass. The contents of the Peligot tubes were transferred to Erlenmeyers and titrated against standard sulphuric acid, using brom-phenol blue as an indicator.

Only qualitative tests were made for the nitrogen of nitrites. Portions of the extract used in the nitric nitrogen determination were made neutral and to them were added 1 c. c. quantities of sulphanilic acid mixture (15). A red color in the extract from the diseased leaves indicated the presence of nitrites. A faint pink appeared in the extract from the healthy material indicating a trace of nitrite nitrogen.

Total hydrolyzable nitrogen and nitrogen distribution in the hydrolyzed portions were determined as follows: Eight-gram samples with 400 c. c. of 20 per cent hydrochloric acid were boiled for 9 hours under a reflux condenser. The contents of the flasks were then filtered and washed with ammonia-free water until free from chlorine. The filtrate and washings of each 8 gm. sample were thereupon made to 2 liters. Total nitrogen was determined on 500 c. c. aliquots by the Kjeldahl-Gunning method. Other 500 c. c. portions were evap-

orated to dryness on the water bath and the acid amide, humin, and diamino acid nitrogen estimated according to Hausmann's nitrogen distribution method.

Protein nitrogen was determined by Stutzer's method (21, p. 105; 22, p. 475). In this method, 1 gm. samples of the dry celery powder were treated in the beaker with water (100 c. c.) heated to boiling and kept on the water bath for from 10 to 15 minutes. Then 2 c. c. of concentrated potassium-alum solution and 15 c. c. (0.45 gm. $\text{Cu}(\text{OH})_2$) of Stutzer's solution³ were added and the mixture stirred thoroughly. On cooling, it was filtered and thoroughly washed with water. The residue and filter paper was transferred to a Kjeldahl flask and the "protein nitrogen" oxidized by the Kjeldahl-Gunning method.

The nitrogen in the filtrate and washings from the $\text{Cu}(\text{OH})_2$ residue was determined by the Kjeldahl-Gunning method and called nonprotein nitrogen. The results for the analysis are nearly the same as those arrived at by subtracting the figures for protein "N" from those for total "N"; they are at least within the error due to sampling which must be considered in all the estimations.

The results of the analyses by the above-described methods are given in Tables I and II.

TABLE I.—Average¹ nitrogen distribution in healthy celery leaves and in leaves affected with "early blight" (*Cercospora apii*)

[Total nitrogen expressed in per cent of oven-dried leaves: Healthy, 4.77 per cent; blighted, 2.94 per cent. Nitrogen distribution expressed in per cent of total nitrogen]

Form of nitrogen	Blighted	Healthy
Nitric nitrogen:		
Scales's Zn-Cu couple.....	5.3	8.7
Schulze-Tieman.....	4.0	6.7
Ammonia nitrogen.....	2.6	1.3
Nitrogen of nitrites.....	(²)	(²)
Total hydrolyzable nitrogen.....	88.3	90.9
Acid amide nitrogen.....	16.5	16.7
"Humin" nitrogen.....	10.6	6.5
Diamino-nitrogen.....	15.5	16.8
Monoamino nitrogen.....	45.6	50.7
Protein nitrogen.....	74.4	68.3
Nonprotein nitrogen.....	25.3	29.7

¹ For data from which these averages are computed see Table II.

² Strongly positive test.

³ Faint trace.

METHODS USED WITH CELERY LEAVES ATTACKED BY SEPTORIA APII

Mature celery leaves of approximately the same age, taken from plants of the Easy Bleaching variety grown on muck soil at Kalamazoo, Mich., were used. The diseased leaflets used for analysis had spots occupying from one-tenth to one-sixth of the leaf area, while those called healthy were apparently free from spot. The samples were dried for two days on cheesecloth over a steam radiator (temperature 40° to 50° C.) and then were pulverized and dried further in an electric oven held at 50° C. Samples were then sealed in jars.

³ Preparation of Stutzer's reagent: 100 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were dissolved in 5 liters of water to which 2.5 c. c. of glycerol were added and then enough NaOH to render the solution faintly alkaline. The precipitate was filtered and washed with water containing 5 c. c. glycerol per liter until free from alkali. The precipitate was then taken up with water containing 10 per cent glycerol and the amount of $\text{Cu}(\text{OH})_2$ per c. c. determined by the following method: 2 c. c. of Stutzer's solution were treated with 2.5 c. c. of concentrated HNO_3 and 25 c. c. of water and then boiled. Treated with 5 c. c. of bromine water, boiled, treated with NH_4OH , boiled, treated with acetic acid, boiled, cooled, 3 gm. KI added, and titrated with $\text{Na}_2\text{S}_2\text{O}_3$.

TABLE II.—Analyses of celery leaves—Healthy leaves contrasted with leaves affected with “early blight” (*Cercospora apii*)

[Data expressed in percentage of the oven-dried celery leaves]

Determinations	Blighted			Average	Healthy			Average
Moisture content:								
Field material.....	79.4	76.3	-----	77.8	80.0	80.16	-----	80.08
Greenhouse.....	75.1	73.8	77.3	-----	84.15	84.25	83.15	-----
	74.2	77.75	77.0	75.86	84.7	84.1	84.4	84.125
Total nitrogen, Kjeldahl-Gunning method (leaves ground).....	2.93	2.92	2.98	2.943	4.78	4.71	4.83	4.773
Nitric nitrogen:								
Scales's Zn-Cu couple method.....	.156	.156	.1601	.1574	.421	.417	.421	.4197
Schulze-Tieman, no-gas method.....	.118	.121	.1195	-----	.3255	.320	.325	.323
			.118	.1191	-----	-----	-----	-----
Ammonia nitrogen, Grafe's method.....	.0783	.081	.0769	.07693	.06045	.0632	.0714	.06456
	-----	-----	.07145	-----	-----	-----	.0632	-----

Nitrogen of nitrites, qualitative tests	Present-good test			Average	Faint trace			Average
Total hydrolyzable “N,” Kjeldahl-Gunning.....		2.601	-----	-----		4.342	-----	-----
Nitrogen distribution, Hausmann's method:								
Acid amide nitrogen.....	0.4843	0.4911	-----	0.4877575	0.7899	.80725	-----	0.7986
“Humin” nitrogen.....	.3160	.3091	-----	.31259	.3229	.30913	-----	.3160
Diamino acid nitrogen.....	.4602	.4533	-----	.456825	.8107	.797	-----	.8038
Monoamino acid nitrogen.....	1.340	1.347	-----	1.3443	2.418	2.428	-----	2.4233
Protein nitrogen, Ritt-hausen-Stutzer method.....	2.174	2.215	2.188	2.1923	3.261	3.281	3.268	3.27
Nonprotein nitrogen “N,” in filtrate after precipitation with Stutzer's reagent CuOH ₂759	.7383	.7383	.7452	1.422	1.415	1.415	1.4173

Analyses of the *Septoria apii* material and the corresponding healthy leaves were made by different methods, for the most part, than those used in the spinach investigations. These methods gave results that were very satisfactory and for convenience of manipulation seem to be desirable. Brief directions for carrying out the various tests are included, but for complete instructions the original articles should be consulted.

METHODS OF ANALYSIS USED ON MATERIAL AFFECTED WITH SEPTORIA APII

In the determination of the total nitrogen the method of Davisson and Parsons (7) was followed. One-gram samples of the material were placed in 500 c. c. Kjeldahl flasks, 100 c. c. of distilled water added, and the suspension shaken for a short period (2 to 5 minutes). Two drops of kerosene, 1 gm. of Devarda's alloy, and 1 c. c. of saturated sodium hydroxide solution were then added; the dissolved nitrates and nitrites reduced to ammonia were caught in the tower containing sulphuric acid (27 c. c. concentrated acid plus 8 c. c. water). The complete reduction of the nitrates was assured by heating the solution to boiling in minimum time and then heating

over a low flame for 20 minutes. At the end of that period the material was again brought to vigorous boiling and the flame removed, the sulphuric acid containing the reduced nitrates being drawn back into the Kjeldahl flask. Twenty-five to 30 c. c. of distilled water were then poured upon the glass beads in the tower and the solution in the flask again brought to boiling; then the flame was removed and the wash water drawn into the flask. This washing was repeated four times, thus assuring complete transference of the sulphuric acid to the digestion flask. The material was then digested according to the Kjeldahl-Gunning method, the potassium sulphate not being added until all the water was boiled off. The digested material was diluted to 175 c. c., 25 c. c. of K_2S solution (40 gm. K_2S per liter) added, the whole made strongly alkaline with saturated $NaOH$ and distilled as in the ordinary Kjeldahl procedure.

For the determination of nitric nitrogen Strowd's method (20) was employed. Eight grams of the material were extracted by shaking vigorously for 5 minutes with 250 c. c. of cold distilled water. The mixture was allowed to stand for 20 minutes and again shaken for 5 minutes. The extract was filtered and determinations made on aliquot portions. The portions were diluted to 250 c. c. in 500 c. c. Kjeldahl flasks and 2 drops of kerosene and 5 c. c. of saturated $NaOH$ added to each. To half of the samples 1 gm. of Devarda's alloy was also added. All samples were connected immediately to a Kjeldahl distillation apparatus, heated over a very low flame for 1 hour and then 150 c. c. distilled over and caught in 50 c. c. of 4 per cent boric acid solution. Care was taken that the controls containing no alloy were distilled at the same rate as those solutions containing the reducing metal. The distillate was titrated against standard acid, using brom-phenol blue as an indicator, as in the Scales method. The difference between the readings of the control samples and those reduced by the alloy is due to the nitrate and nitrite nitrogen.

The determination of ammonia nitrogen was made by the aeration method of Folin (10, p. 499-500). One-gram samples of the healthy and diseased materials were placed in the aeration tubes and to each were added 10 c. c. of water, 1 c. c. of saturated potassium oxalate and 0.5 gm. of anhydrous sodium carbonate. The aeration was effected by means of a water pump, the rate being determined by the tendency of the suspensions to foam over. Aeration was continued for 12 hours. The ammonia was caught in 4 per cent boric acid and titrated as described.

Only a qualitative test for nitrite nitrogen was made. Portions of the extract used in nitric nitrogen determinations as in Jodidi's procedure for spinach were made neutral and to them was added 1 c. c. quantities of the sulphanilic acid mixture. No nitrite was found by this method.

A different method of extraction than that used by Jodidi's procedure was then employed for the test. Ten grams of celery were extracted for 5 minutes with 150 c. c. of distilled water and the infusion thus obtained was filtered through fine Swedish filter paper. The filtrate which had a brown color was partially clarified by shaking for 2 minutes with 10 gms. of Lloyd's reagent with subsequent filtering; 25 c. c. of this filtrate was treated with 3 gms. of Lloyd's

reagent and filtered again; 15 c. c. of this last filtrate was diluted to 50 c. c. in a Nessler tube and tested for nitrite with the sulphanilic acid mixture (2, p. 23). The extract from diseased material gave a very strong pink, showing presence of nitrites, whereas a similar procedure with healthy material and with blanks gave no reaction. To determine total hydrolyzable nitrogen, samples of 4 gms. each of the dried powder in 200 c. c. of 20 per cent hydrochloric acid were boiled for 9 hours in 500 c. c. Kjeldahl flasks under a reflux condenser. The "humin" nitrogen was then filtered off and washed. The filtrate and washings were then made up to liter. Total hydrolyzable nitrogen was determined by Kjeldahlizing 250 c. c. portions of the filtrate.

NITROGEN DISTRIBUTION IN THE HYRDOLYZED MATERIAL

The filter paper bearing the "humin" nitrogen precipitate was returned to the Kjeldahl flasks used in refluxing, and the digestion and distillation carried out by the Kjeldahl-Gunning method. The nitrogen recovered was called "humin."

Acid-amide nitrogen was determined by using aliquot portions of the hydrolyzed material which were made slightly alkaline with strong alkali, and the acid-amide nitrogen forming ammonia, together with the ammonia nitrogen present, was aerated off as in the ammonia nitrogen determinations.

Total amino nitrogen was determined by use of the Van Slyke micromethod. To avoid the interference of the presence of ammonia and amido nitrogen, the aerated solutions from the acid-amide nitrogen determinations after neutralization with sulphuric acid were used.

The protein nitrogen was determined by the method of Stutzer in which 1 gm. samples of the dry celery powder were treated as described in the work with the Cercospora material.

The figures for nonprotein nitrogen were obtained by computation.

The results of the analyses by the above described methods are given in Tables III and IV.

TABLE III.—Average nitrogen distribution in healthy celery leaves and in leaves affected with Septoria leaf spot (*Septoria api*) ¹

[Total nitrogen expressed in percentage of oven-dried leaves: Healthy, 5.10 per cent; diseased, 4.38 per cent. Nitrogen distribution expressed in terms of total nitrogen]

	Diseased	Healthy
Nitric nitrogen.....	5.7	5.8
Ammonia nitrogen.....	1.3	0.3
Nitrogen of nitrites.....	(²)	(³)
Total hydrolyzable nitrogen.....	84.9	88.8
Acid amide.....	11.8	13.5
"Humin".....	10.9	9.6
Total amino nitrogen.....	43.5	47.1
Protein nitrogen.....	72.6	67.8
Nonprotein nitrogen.....	27.1	32.1

¹ For data from which these averages are computed see Table IV. ² Present. ³ Absent.

TABLE IV.—Analyses of celery leaves.—Healthy leaves contrasted with leaves affected by *Septoria leaf spot* (*Septoria apii*)

[Data expressed in percentage of oven-dried celery leaves]

Blighted leaves				Average	Healthy leaves			Average
Total nitrogen.....	-----	4. 378	4. 374	4. 376	5. 037	5. 15	5. 12	5. 102
Nitric nitrogen.....	-----	. 2501	. 2501	. 2501	. 285	. 310	-----	. 297
Ammonia nitrogen.....	. 0621	. 0552	. 0621	. 0598	. 0207	. 0138	. 0138	. 0161

Nitrite nitrogen	Positive test			Average	Negative test			Average
Total hydrolyzable nitrogen.....	-----	3. 705	3. 726	3. 715	4. 485	4. 581	-----	4. 533
Distribution of above:								
Acid amide ¹ 5175	. 5175	. 5175	. 5175	. 69	. 69	-----	. 69
" Humin ".....	. 479	-----	-----	. 479	. 4623	-----	-----	. 4623
Total Amino.....	1. 897	1. 911	-----	1. 904	2. 423	2. 39	-----	2. 406
Protein nitrogen.....	3. 14	3. 22	3. 20	3. 18	3. 46	3. 45	3. 47	3. 46
Nonprotein nitrogen.....	1. 236	1. 156	1. 176	1. 189	1. 642	1. 652	1. 632	1. 642

¹ Ammonia nitrogen not subtracted.

DISCUSSION

A comparison of the figures for the two leaf diseases of celery shows that there is in the case of each disease a lower total nitrogen content in the diseased than in the healthy leaves. This is the striking thing in the analysis. Whereas the difference between healthy and diseased leaves is somewhat greater with the *Cercospora apii* material than with *Septoria apii*, this variance is not believed to be significant since the extent of the diseased tissue was greater with the *Cercospora apii* material than with that from *Septoria apii*. The consistently lower total nitrogen content is the important consideration.

There is a lower nitric nitrogen content in diseased leaves. The difference is not significant in the case of *Septoria apii* material, but is pronounced in the case of *Cercospora apii*.

Ammonia nitrogen, in the case of both the *Cercospora apii* and the *Septoria apii* tests, was greater in the diseased material than in the corresponding healthy material. While the differences between healthy and diseased material is less in the case of *Septoria apii* than with *Cercospora apii*, this is not to be taken as a characteristic difference always to be expected. It is rather to be referred to the percentage of diseased material present in the samples. It has commonly been noticed that with leaves extensively affected with *Septoria apii* an ammonia odor is very noticeable.

With *Cercospora apii* material, nitrite was definitely shown to be present by using Jodidi's technique. It was only faintly evident in the healthy, control material. Nitrite could not be so demonstrated with *Septoria apii* material, although tested repeatedly. By a different method of extraction, however, abundant evidence of presence of nitrites was obtained.

With *Cercospora apii* material, the diamino acid content was determined, the monoamino nitrogen being obtained by difference. The healthy leaflets showed the greater per cent of these compounds. With *Septoria apii*, total aminoacid nitrogen was determined, and here also the amount in the healthy leaflets exceeded that of the

diseased leaflets. With diseased leaves, the protein nitrogen was greater than that present in the corresponding healthy control leaflets, in the case of both fungi. The percentages of humin nitrogen was noticeably greater in the diseased leaves.

This outline of the relative nitrogen constituents for healthy and diseased celery shows that with these two parasitic diseases the results of analyses follow closely those of spinach mosaic and those of the unknown cabbage disease to which reference has been made.

TABLE V.—*Deviation of diseased material from healthy material—Comparison of celery diseases with results obtained by Jodidi et al. with spinach and cabbage*

[Hot water extract of spinach and cabbage was used for the acid amide, "humin," diamino, and monoamino tests. The results are expressed in percentage of total nitrogen]

	Celery		Spinach mosaic	Cabbage unknown disease ¹
	Cercospora apii	Septoria apii		
Nitric nitrogen.....	-3.4 (-2.7)	-0.1	² -1.22	-6.42
Ammonia nitrogen.....	+1.3	+1.0	³ +0.63	
Nitrite.....	(+)	(+)	² (+)	(+)
Total hydrolyzable nitrogen.....	-2.6	-3.9		
Acid amide.....	-0.2	⁴ -1.7	² -2.89	⁴ -7.19
"Humin".....	+4.1	+1.3	² +0.94	+0.81
Diamino.....	-1.3	-3.6	² -2.33	-4.04
Monoamino.....	-5.1			
Protein nitrogen.....	+6.1	+4.8	³ +9.29	+21.72
Relation of total nitrogen of diseased to healthy material...	61	83	² 77	77

¹ Figures computed from Tables II, III, and IV (leaves), Jodidi (12).

² Figures computed from sample A, Table V, p. 1891, Jodidi (12).

³ Figures computed from Tables III and IV, Jodidi et al. (13).

⁴ Plus ammonia nitrogen.

It will be noted that in every case the deviation of diseased material from healthy is of the same character. Jodidi, because of the similarity of the chemical picture of diseased cabbage and spinach, deduced that the cabbage disease was of mosaic nature. The results of the analyses here presented show that such a deduction is unwarranted.

The results here obtained seem best interpreted from the point of view of metabolism of fungi. A parasitic fungus maintains itself by establishing a food and water relation with a host. From the nitrogenous material of the host it builds its own kinds of proteins. Since Nägeli's classic researches (18), it has commonly been accepted that the splitting off of ammonia is the course of protein decomposition by microorganisms. The presence of ammonia in cultures of fungi on richly nitrogenous media has often been demonstrated. Wehmer (26) in cultures of *Aspergillus niger* on peptone solution obtained marked formation of ammonia. Further evidence of the formation of ammonia from proteins have been given by many others with a wide range of organisms (4, p. 310). But there are intermediate steps in the process and these are poorly known. The work of Butkewitsch (6) may be cited as throwing some light on this process. *Aspergillus niger*, *Penicillium glaucum*, *Mucor racemosus*, and *Rhizopus nigricans* were shown by him to split off not only ammonia from peptone and fibrin, but also aminoacids, leucin, and tyrosin. (See also Klotz, 14.) These products were in turn decomposed. From the work of various investigators it has been shown that the process is

enzymic and in various organisms, proteases of various types and enzymes which split amide bodies have been demonstrated. (Waksnan, 24, 25.)

In the metabolism of fungi and bacteria the reduction of nitrates to nitrites and to ammonia is of common occurrence (11).

The loss of nitrogen in the celery leaves, through the agency of the two fungi, is believed to be not essentially different from the loss of nitrogen from a medium in which the organisms are growing. In short, the fungus uses the nitrogen compounds of the leaf tissue for food. Behrens (3) quotes the work of Müller-Thurgau who had noted the lower nitrogen content of the juice from rotted grapes as compared with juice from sound grapes. In another series of tests Müller-Thurgau grew pure cultures of *Botrytis* upon the grape must and found that the nitrogen content became only one-fifth of the initial amount after 21 days of culturing.

It would seem that the effect of the parasites in the celery disease is to bring about a loss of total nitrogen. The mechanics of the process in cases of parasitism is not known and the use of the term "denitrification" in this connection is misleading. Because of the presence of a trace of nitrite in diseased tissue Jodidi (12) has suggested that the action of this compound upon the amino acid group liberated free nitrogen, by a reaction practically similar to the Van Slyke reaction. He presents no evidence to support this suggestion.

The loss of nitrogen as commonly brought about by organisms seems to be by the splitting off from proteins ammonia which is later liberated. Reasoning from studies on nitrogen metabolism of fungi in cultures the following processes may be assumed to take place (Klotz, 14): As the strictly carbonaceous food components, such as the carbohydrates, of a lesion become exhausted, probably the nitrogen compounds, as the amino acids, peptones, and proteins must be decomposed to furnish the parasite with carbon requisite for metabolism, especially respiration, or the organism may draw upon its own cell reserves for the nitrogen and carbon to continue life processes. Where the proportion of the nitrogen to the nonnitrogenous complex of either the substrate or of the mycelium itself is greater than is required in the metabolic processes, in protein synthesis (or protoplasmic repair) the excess nitrogen appears as NH_3 and is lost as such. The basis for the above suggestions is to be found in the behavior of fungi in culture since it was found that ammonia was never formed in or excreted into the culture fluid when sugar was present. The disappearance of carbohydrate from the culture medium was found to be synchronous with the appearance of ammonia and with the beginning of autolysis of the fungus. Ammonia was found to be the chief nitrogenous product of the splitting of peptone of the medium in the absence of another carbon source, but in the presence of dextrose NH_3 was reassimilated.

Loss of total nitrogen in the later stage of necrotic diseases is therefore to be expected if the relation to the host is comparable to the relation of a fungus to a culture medium.

Assuming that this is the case in typical parasitic diseases, is one justified in concluding that whenever there is a condition where lower total nitrogen occurs that one is confronted with the work of parasites?

The similarity of the chemical picture of disease in the spinach and in the celery points very strongly to a parasitic agency being at work in the spinach. But it must be borne in mind that it is not known at all what effects these various derangements of normal nutrition produce on plant growth, and exactly as some of these conditions produce spots, lesions, overgrowths, etc., which simulate the diseases brought about by parasites, so a disturbed physiology from other than parasitic agency may give a chemical aspect which simulates more or less closely the condition which accompanies these two diseases caused by parasitic fungi. It would seem that the effects which environmental conditions produce would be largely of a repressive nature, depressing, for example, the total nitrogen production, and such a condition might also be expected with hypoplastic diseases. Necrotic diseases of the types studied, on the other hand, are destructive, reducing that which has already been produced.

With such a criterion, the analyses given when compared with those of spinach go to emphasize that in the case of an infectious disease, such as spinach mosaic is known to be, one may be dealing with a parasite whose effect upon the host is not dissimilar to known parasites, but the similarity of chemical relations alone does not permit judgment in this matter. As far as the cabbage disease is concerned, its infectious nature has not been shown and the evidence at hand does not warrant judgment on purely chemical grounds, as to its parasitic nature. Surely the similarity of chemical analyses is not proof that the cabbage disease is a mosaic disease.

The interpretation of disease production on the basis of the parasite simply establishing a food and water relation with the host opens the question whether disease production by parasites in general is to be interpreted on such simple grounds. Is the living together of host and invader in all types of parasitism, especially the higher types, merely a food and water relation and is the matter of specialization of a parasite upon a species or upon related species determined by the ability of the parasite to invade, grow, and to wrest food from the reserve stuffs of the host?

The data here presented deal with organisms of not a very high specialization in parasitism. These organisms simply produce a necrotic condition in the host. There is no stimulation of the host nor any evidence of tolerance by the tissues invaded. Whether toxic substances are produced by the invaders is not known. These two organisms, though readily grown in pure cultures on ordinary laboratory media of complex nature or on synthetic media of simple salts and sugar, have not been found to be omnivorous in their parasitic habit. Both, on the contrary, are extremely selective in their parasitism, attacking only the celerylike plants (*Apium graveolens*).

The analyses seem to show that the striking thing in the disease production is the mere feeding on the host tissue, the disintegrating of protein in the processes of metabolism. It would seem plausible to suggest that with the necrosis-producing parasites at least, the ability to appropriate food is a very important factor in parasitism. The fungus must first be able to invade and tolerate the conditions within the host, not being walled off or killed by acid or some cellular product. After these barriers are passed, the degree of disease production may largely be correlated with the ability to attack the

food substance of the host, especially the proteins. As in higher plants and animals, the breaking down of proteins and other complex nitrogenous compounds by fungi is accomplished by proteolytic enzymes. These may arise from the host cells themselves in the form of autolytic enzymes which may be released for action by the conditions which the parasite sets up, or the invader may furnish the proteolytic enzymes. By a study of these conditions and especially by studies on the fungous proteases one shall be approaching closely toward a solution of the problem of selective parasitism. Here also lies the domain of immunity to disease, and biochemical studies to determine the nature of immunity must logically turn in this direction.

SUMMARY

Comparative analyses of healthy and diseased celery leaves affected with *Cercospora apii* and *Septoria apii* have been made and it has been found that in the leaves affected with these necrotic diseases there is a lower percentage of total nitrogen in the diseased than in the healthy tissue. Nitrites are present in the diseased material. A comparison of the nitrogenous compounds present show in per cent of total nitrogen, greater ammonia, greater humin, greater protein, less hydrolyzable, less acid amide, less basic and less nonprotein nitrogen in the diseased than in the healthy tissues.

It is believed that these results can best be explained as due to the decomposition of the host by the parasite in a simple food relation.

The existence of a similar chemical picture in the spinach mosaic and in the unknown cabbage disease studied by Jodidi and his associates does not warrant the assumption that the spinach mosaic is of parasitic nature, although the results are very suggestive, nor can the cabbage disease be diagnosed on the basis of mere chemical analyses as a true mosaic.

The importance of the nitrogen metabolism of parasitic fungi is stressed as a possible explanation of selective parasitism and as a point of attack in immunological research.

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No. 4

STEAM AND CHEMICAL SOIL DISINFECTION WITH SPECIAL REFERENCE TO POTATO WART¹

By N. REX HUNT, *Pathologist*, and F. G. O'DONNELL and RUSH P. MARSHALL,
*Plant Quarantine Inspectors, Federal Horticultural Board, United States Department of Agriculture.*²

INTRODUCTION

Soil disinfection studies were begun in 1919 in order to find a method by which the potato-wart disease caused by *Synchytrium endobioticum* (Schilb.) Perc. could be eradicated from infected soil. At that time little was known of the susceptibility of important potato varieties grown in the United States or of the probable rate of spread of the disease. With a total known infected area of only about 100 acres in Pennsylvania, Maryland, and West Virginia (fig. 1) largely in the mountainous coal-mining regions, treatment of the entire area would have been feasible if a cheap, effective method of treatment had been found and the disease had proved destructive to a large number of our commercial varieties. It should be borne in mind that any method of soil treatment that would kill the thick-walled wart sporangia would almost certainly be too expensive to use even on small outlying areas unless it insured actual extermination of the wart organism—nearly perfect control would not justify such heavy expenditures.

In order to determine the susceptibility of potato varieties and of related plants and to study the behavior of the disease in America, as well as to test possible methods of eradication, a field station was established at Freeland, Pa., in 1919 and work was carried on during that and several succeeding years.

Fortunately a majority of our commercial potato varieties seem to be immune or highly resistant to the potato-wart disease and the disease appears to spread rather slowly. Quarantines and the growing of immune varieties in infected areas seem likely to eradicate the disease in time (20, 21).³ Although a number of soil treatments were used with apparently entire success in the eradication experiments, the development of some fundamental principles underlying the successful use of heat and, more particularly, of chemicals in soil disinfection, and the presentation of the supporting data constitute the more important parts of this report.

¹ Received for publication June 6, 1924; issued September, 1925.

² These studies were made as part of a general cooperative project on potato wart between the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, the Federal Horticultural Board, United States Department of Agriculture, Pennsylvania State College, and the Pennsylvania State Department of Agriculture. Authorship of Part II should be credited to Hunt and O'Donnell, of Part IV to Marshall.

³ Reference is made by number (*italic*) to "Literature cited," p. 363.

PART I.—THE EFFECT OF STEAM AND CHEMICAL SOIL TREATMENTS ON THE OCCURRENCE OF POTATO WART

Synchytrium endobioticum has no mycelium, producing only sporangia and swarm spores. The latter infect potato stolons and tubers, usually only at the eyes, producing the cauliflower-like outgrowths or excrescences commonly called "warts." These warts soon decay, leaving the numerous sporangia to become incorporated in the soil and to spread by cultivation, water and air currents, tubers, tools, the feet, or other agents (22). According to apparently authentic reports the sporangia may remain viable in the soil for many years. Soil disinfection, if practicable and effective, would immediately remove the possible sources of infection.

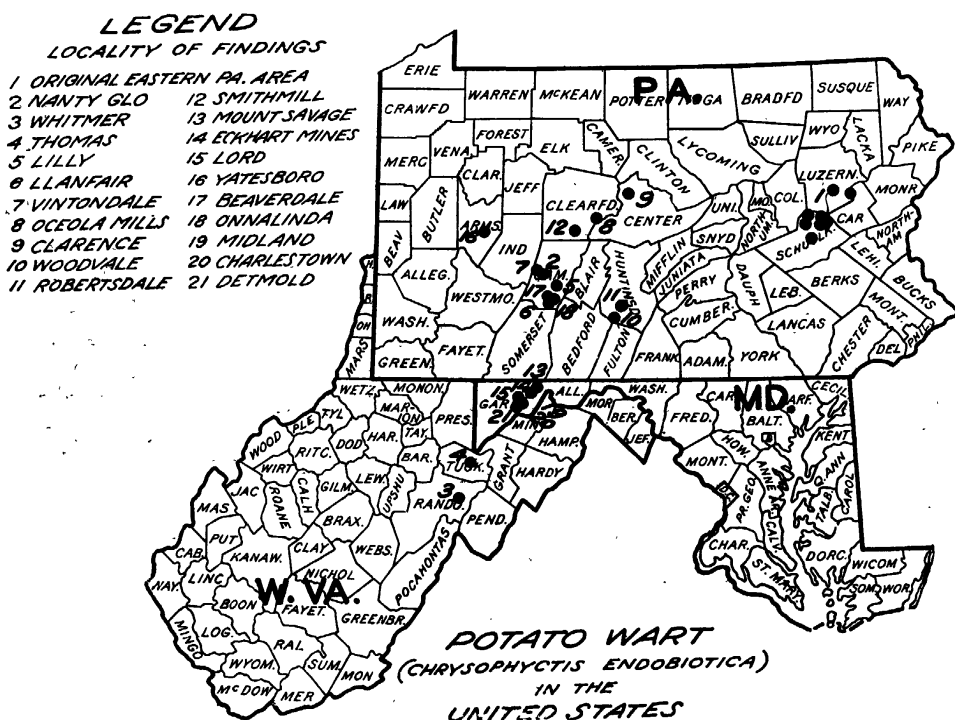


FIG. 1.—Known distribution of potato wart in the United States in 1921. Field experiments were carried out near Freeland, Pa., in the large infected area in eastern Pennsylvania

The soil disinfection experiments carried out have followed two main lines of attack: (1) The use of steam by the inverted-pan method, either alone or in conjunction with a chemical; (2) the use of chemicals.

THE STEAM-PAN TREATMENTS

HISTORICAL

Gilbert (4) seems to have been the first to try the inverted steam-pan method of soil disinfection for controlling a plant disease. In testing methods of disinfecting tobacco-seed beds for the control of root rot (*Thielavia basicola*), Gilbert used the inverted steam-pan on three plots, one "treated for a half hour after the soil had reached a temperature of 200° F. as indicated by a soil thermometer inserted 6 to 8 inches below the surface of the soil, 'a second' was treated one hour at a temperature of 175° 'and the third' one and one-half hours

at a temperature of 150°. The second of these plots gave the best results, being almost entirely free from disease." On the basis of his experiments Gilbert recommends that soil to be disinfected should be spaded up and thoroughly pulverized and steam applied by the inverted-pan method. "The steam should be kept at as high a pressure as possible, 80 to 100 pounds being best, and the treatment should continue for one to two hours, depending on the pressure maintained."

Numerous investigators have obtained results very similar to those of Gilbert, and the use of the steam-pan treatment of seed beds for the control of soil-borne diseases has become common practice in many places. Formaldehyde drenching has also been a common treatment for seed beds. The strength and quantity of formaldehyde solution recommended varies considerably. Gilbert recommends "1 part of commercial formalin to 150 to 200 parts of water, three-fourths to 1 gallon of this solution being used to the square foot of bed space."

Both steam and formaldehyde treatments had been used with such success in this country that Kunkel and Broadbent, who in 1919 experimented with possible methods of exterminating potato wart, undertook a retesting of these methods, notwithstanding the reported lack of success with them in England. They also tried combinations of the two treatments. In 1919, field work on this project was carried out by Broadbent⁵ in heavily infected soil near Freeland, Pa. Broadbent's data show that his steam treatments varied from 25 minutes at 85 pounds gauge pressure to 175 minutes at 105 pounds pressure. No wart infection appeared in plots treated for 85 minutes at 90 pounds pressure, but occurred in a number of plots steamed 70 minutes. Plots which were sprinkled with 0.41 per cent (or stronger) formaldehyde at the rate of 0.59 pint per square foot and immediately steamed for 30 minutes at 90 pounds pressure were free from wart. The treatments with formaldehyde alone were unsuccessful.

1920 STEAM-PAN TREATMENTS

Steaming operations were begun at Freeland, Pa., on May 26, 1920. Thirty-one plots were treated up to and including June 8. The remaining seven plots were treated during the period June 25 to July 2. The garden used for the steam-pan experiments in 1920 adjoined the one used for similar work in 1919, the latter in turn adjoined the garden from which the disease was first reported. Freeland is at the southern boundary of the glacial advance and the soil contains elements of the Volusia and DeKalb types. Both are largely silt loams with a tendency to be acid. The gardens of the coal miners of that locality are largely a conglomeration of soil, ashes, coal-dust, cinders, and débris of any kind, and the steam-treated garden was no exception.

The source of steam for the experiments of both years was a large high-pressure (135 to 140 pounds) main used for pumping, hoisting, and other work about the mines. Steam was conducted nearly 200 feet from the main through a $\frac{3}{4}$ -inch pipe, a 20-foot length of steam hose serving to connect this pipe with the steam-pans. The steam-pans were those used in 1919, one covering 5 by 9 feet and the other 6 by 9 feet. These are merely shallow open boxes; the sides are made of 2 by 6 inch material from the lower edge of which an iron

⁵ Unpublished report of field work in soil disinfection to exterminate potato wart, 1919.

strip projects to insure a steam-tight contact with the soil. The top consists of two layers of thin tongue-and-groove boards with a layer of tar paper between. Handles are bolted to the sides. The steam inlet is centrally located in the top of the pan, and a metal plate fastened just below the opening serves to deflect the steam in all directions parallel to the soil surface. A steam-pressure gauge was attached to the steam supply pipe about 40 feet from the pan. The pressure was read with the steam to the pan shut off. The duration of steaming is expressed as the time elapsing between the opening and closing of the valve at the gauge. The pan was left in place for 30 minutes after steam was shut off, thus prolonging the heating effect. By using electric resistance bulbs the rise and fall of temperatures at different depths in the soil under the steam pan was followed. A summary of these data and of the conditions under which the treatments were carried out is presented later. It seems likely from later tests that the amount of steam supplied to the steam pan was not accurately measured by the gauge pressure. The temperatures obtained were not always uniform. This may explain the lack of complete disinfection in some of the plots which were steamed for long periods.

When the pans were moved they were set down so as to overlap by at least 6 inches the area previously steamed. Usually two pan areas which were similarly treated constituted a plot. In a few cases, one or three pan areas made up a plot. As soon as the steaming of a plot was completed, a trench a foot wide and 3 inches deep was carefully dug around it. Stakes were then driven into the soil at each corner. Boards were nailed to these to form sides which projected above the soil level and slightly below the bottom of the trench. The trench and board frames were provided to prevent contamination of the plots by surface water. A 2-foot wire-netting fence was attached above these base boards and a wire-netting top was fitted on in order to exclude birds and quadrupeds which might otherwise contaminate the plot. Great care was taken to have boots, tools, lumber, and other supplies used about the plots sterile so that no infected material reached the steamed soil during the progress of the work. The wind blew outside soil across the steam plots on one or two occasions. It was found necessary to sprinkle the surrounding area lightly with water to prevent this. These experiments in 1920 were planned to retest the steam and formaldehyde treatments which the results obtained in 1919 indicated to be of greatest promise. In view of the striking results obtained in 1919 by the application of dilute solutions of mercuric chloride, certain plots received a combination of mercury drench and steam treatment.

The steam-pan treatments in 1920, using steam alone, varied from 70 to 115 minutes, with the gauge showing 90 to 95 pounds pressure, and from 12 to 25 minutes at 140 pounds pressure. The steam-pan treatments which followed the application of formaldehyde at the rate of $\frac{1}{2}$ pint per square foot ranged from 25 to 40 minutes of steaming at 90 to 95 pounds pressure. The steam-pan treatments which followed the application of mercuric chloride each lasted for 25 minutes with steam at 90 to 95 pounds gauge pressure. Mercuric chloride was applied at the rate of 80 c. c. of a 1 to 20,000 solution to 80 c. c. of a 1 to 500 solution per square foot, and in one instance at the rate of 160 c. c. of a 1 to 400 solution per square foot.

RESULTS OF STEAM-PAN TREATMENTS

RESULTS IN 1920

Through an error on the part of the seed house which furnished them, the potatoes used in planting most of the plots in 1920 were of a wart-immune variety and consequently very meager results were obtained. Nine plots were, however, planted with susceptible varieties. Of these, five became infected with wart, as did also two plots which were left untreated as controls. Two plots in which wart developed had been treated with 1 per cent formaldehyde ($\frac{1}{2}$ pint per square foot) followed by steam at 90 to 95 pounds pressure for 25 minutes; one with 1.5 per cent formaldehyde followed by steam for 40 minutes; and one treated with steam alone for 12 minutes at 140 pounds pressure. The four plots which were free from wart at harvest had been treated as follows: One plot with 160 c. c. per square foot of a 1 to 400 solution of mercuric chloride followed by steam for 25 minutes; one with $1\frac{1}{2}$ per cent formaldehyde and steamed for 25 minutes; one with steam alone at 140 pounds pressure for 25 minutes; and one with steam alone for 75 minutes at 90 to 95 pounds pressure. The results practically eliminated the possibility of using a short steaming period following treatment of the soil with formaldehyde, since four of the five plots receiving such treatments and planted to susceptible varieties were found to have produced warted potatoes.

RESULTS IN 1921

In 1921 the plots given steam-pan treatments in 1920 were planted with varieties of potatoes known to be susceptible to wart. Figure 2 presents a diagram showing the treatments given, the relationship of the plots to each other, and indicates the occurrence of wart in 1921. Table I summarizes the results obtained.

Three of the 6 plots given 85 minutes or more of steaming at 90 pounds pressure showed wart, as did a large part of the steam-formaldehyde plots and all of the steam-mercuric chloride plots. Steaming for 85 minutes should heat every wart spore in the soil to a temperature of 90° C. or more and keep it hot for some minutes. These results indicate that the potato wart organism is very resistant to heat. It may be that only the wart spores in the upper 4 or 5 inches of soil were killed by the treatments. Potatoes were planted the first year without stirring the soil to more than this depth, whereas the plots were spaded to a depth of 7 or 8 inches before planting the second season. This may explain in part at least the difference between the results Broadbent obtained from planting the same year the treatments were given, and the results cited above, which were derived principally from plantings made the year following treatment. This view is borne out by the fact that of the nine plots treated in 1920 and planted to susceptible varieties in 1920 all but the one given 75 minutes of steaming showed wart in 1921, whereas in 1920 four of these plots were wart free.

One of the plots which was steamed for 85 minutes and was free from wart at harvest lay between two plots which showed over 60 per cent of wart-infected plants. This would indicate that under favorable conditions this treatment is effective, even in badly infected soil. It will be noted that steaming at the maximum pressure

STEAM GARDEN

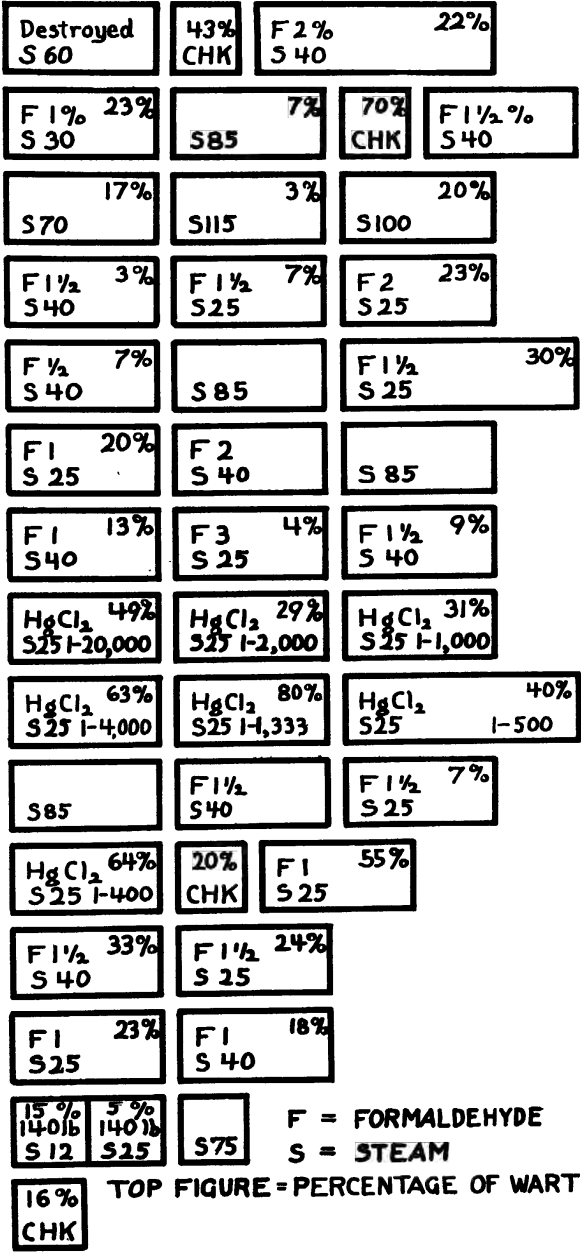


FIG. 2.—Diagram of the plots treated with the steam pan in 1920, showing relative size and arrangement of plots. The length of steaming periods is shown in minutes (e. g., S 60=steam allowed to flow into pan for 60 minutes). The steam pressure was 90 to 95 pounds, except for the two plots where 140 pounds pressure is indicated. The steam pan was removed 30 minutes after steam was shut off. Where 1/2 pint of formaldehyde solution per square foot was applied previous to steaming its strength is indicated (e. g., F 1 1/2 or F 1 1/2 %=1 1/2 per cent of commercial formaldehyde). Mercuric chloride solutions were used at the strengths indicated. The applications were at the rate of 80 c. c. per square foot except that the 1 to 400 solution was applied at the rate of 160 c. c. per square foot. The upper numeral (when present) is the percentage of the hills of potatoes showing wart in 1921. (Building operations caused the destruction of one plot before any results were secured)

TABLE I.—*Presence of wart in 1921 following steam and combination treatments in 1920 ^a*

Wart absent					Wart present						
Formaldehyde		Steam		Plots	Formaldehyde		Mercuric chloride		Steam		Plots
Strength	Amt. per sq. ft.	Pressure	Time		Strength	Amt. per sq. ft.	Strength	Amt. per sq. ft.	Pressure	Time	
<i>Per cent</i>	<i>Pints</i>	<i>Pounds</i>	<i>Minutes</i>	<i>Number</i>	<i>Per cent</i>	<i>Pints</i>	<i>Per cent</i>	<i>C. c.</i>	<i>Pounds</i>	<i>Minutes</i>	<i>Number</i>
		90 to 95	85	3					90 to 95	115	1
		90 to 95	75	1					90 to 95	100	1
2	1½	90 to 95	40	1					90 to 95	85	1
1½	½	90 to 95	40	1					90 to 95	70	1
									140	25	1
									140	12	1
					1½	½			90 to 95	40	4
					1	½			90 to 95	40	2
					½	½			90 to 95	40	1
					2	½			90 to 95	30	1
					1	½			90 to 95	30	1
					3	½			90 to 95	25	1
					2	½			90 to 95	25	1
					1½	½			90 to 95	25	4
					1	½			90 to 95	25	3
							1 to 400	160	90 to 95	25	1
							1 to 500	80	90 to 95	25	1
							1 to 1,000	80	90 to 95	25	1
							1 to 1,333	80	90 to 95	25	1
							1 to 2,000	80	90 to 95	25	1
							1 to 4,000	80	90 to 95	25	1
							1 to 20,000	80	90 to 95	25	1

^a Commercial formaldehyde considered as 100 per cent. One plot was destroyed during winter 1920-21. English weights and measures were used when metric apparatus was not available or whenever more convenient for any reason.

(135 to 140 pounds) carried by the mains was tried. Steam at this pressure heats the soil very rapidly but the treatment is too difficult to carry out. The 12-minute steaming period was due to the bursting of the hose. An ordinary boiler can not maintain such high pressure. The combination of formaldehyde with short periods of steaming did not prove effective. Formaldehyde applied at the rate of one-half pint per square foot does not penetrate the soil more than about an inch. Steaming for 40 minutes at 90 pounds pressure can not be depended on to sterilize deeper than 3 to 4 inches. As noted before, this treatment might result in a clean crop the first year, before the deeper-lying spores are brought to the surface by cultivation. As will be noted in Table I every plot treated with both steam and mercuric chloride was found to have warted plants. The quantity of mercuric chloride solution applied penetrated only a fraction of an inch and the steam treatment added would not be effective to a depth greater than 2 to 3 inches.

The results of the 1920 steam-pan experiments seem to eliminate the steam-formaldehyde and the steam-mercuric chloride treatments, inasmuch as they were too expensive to use unless they insured absolute eradication. The results also cast grave doubts on the dependability of steaming alone for 85 minutes at 90 pounds pressure. The steam-pan treatments as carried out were not effective. The failure of the longer treatments was probably due to the fact that the method of controlling the amount of steam supplied was not accurate, as determined later. However, much of the ground could not be readily treated by the steam-pan method.

Potato wart has been found in over 800 gardens. These vary in size from a few square feet to one-fourth of an acre. Furthermore, the gardens are usually irregularly shaped and are likely to be rendered more difficult to treat by the presence of walks, outbuildings, trees, clothesline posts, ditches, or rock outcroppings. It is practically impossible to treat all the soil next the fences and buildings. In considering the possibility of exterminating potato wart it must be borne in mind that the pathogene is undoubtedly present to a greater or less extent in soil surrounding the gardens in which its presence has actually been established. Spores are easily carried on the feet or on implements from one part of the yard to another and also to adjacent streets, alleys, and yards. The disease was often particularly severe along rear walks where potato peelings had been thrown from the kitchen door. Diseased potatoes were sometimes stored in earthen-floored cellars. Besides these practical difficulties in the use of steam-pan treatments, it is a rather slow and expensive method. Because of the difficulties involved in steam-pan treatments, it seemed desirable to restrict further studies on soil disinfection to tests of chemicals, including a study of the principles underlying their use.

DISINFECTION OF SOIL BY VARIOUS CHEMICAL TREATMENTS

HISTORICAL

The use of chemicals by prior workers for sterilizing soil has, like that of steam, been practically limited to the treatment of greenhouse benches or to seed beds. In either case control rather than eradication was the object sought. For seed beds particularly, only surface sterilization was necessary, as in many cases the control of damping off was the principal objective. Halstead (6) carried out an extensive series of experiments by treating soil with ashes, benzine, Bordeaux, calcium carbonate, carbon bisulphide, copper sulphate, corrosive sublimate, creolin, cupram, formalin, gas lime, kainit, kerosene, lime, manure, oxalic acid, sodium carbonate, sodium chloride, sulphate of ammonium, sulphate of potassium, sulphide of ammonium, sulphide of potassium, sulphur, and sulphuric acid. These experiments showed that sulphur was a valuable preventive of potato scab; that it was a preventive of soil rot in sweet potatoes, especially when used with kainit; and that air-slaked lime was a practical remedy for club root in turnips. The other chemicals failed to show promise as soil fungicides. As control, not extermination, of the diseases was the object of the experiments, the amount of chemical used was relatively small in all cases. The maximum applications of mercuric chloride, for example, were 1 gram per square foot dry, or 1 pint of a 1 to 1,000 solution; of sulphur $\frac{2}{5}$ ounce; of calcium carbonate 1 ounce; and of kerosene $\frac{1}{25}$ ounce per square foot. Only surface or partial sterilization was possible with the amounts used.

For the control of tobacco root rot, formaldehyde drenching was found by Gilbert (4) to give reasonably satisfactory results, though less effective as well as more expensive than the steam treatments tried. Hartley and others (7, 8, 9) have done considerable work on methods of controlling damping off among coniferous seedlings. They found that the chemicals which gave the best results depended on the character of the soil and the organism or organisms involved.

Efforts of European investigators to control or eradicate potato wart by soil-disinfection methods have failed. Among the chemicals

tried were formaldehyde, lime, bichloride of mercury, and sulphur. Malthouse (12, 13, 14) and others in England, and Schaffnit and Voss (19) in Germany tried a wide range of fungicides in seed and soil treatments without finding any method that gave consistent control. Malthouse, for example, found that copper sulphate used as a summer dressing almost eradicated the disease in 1909, but failed to check it the following year. Eriksson (3) reported successful control of potato wart with 1 per cent formaldehyde used at the rate of 1 liter per square meter. Experiments in England (1) based on the results of Eriksson, but on a larger scale, failed to show any control where 1 per cent formaldehyde was used at the rate of 2 gallons per square yard. Every hill, in both treated and untreated soil, showed wart.

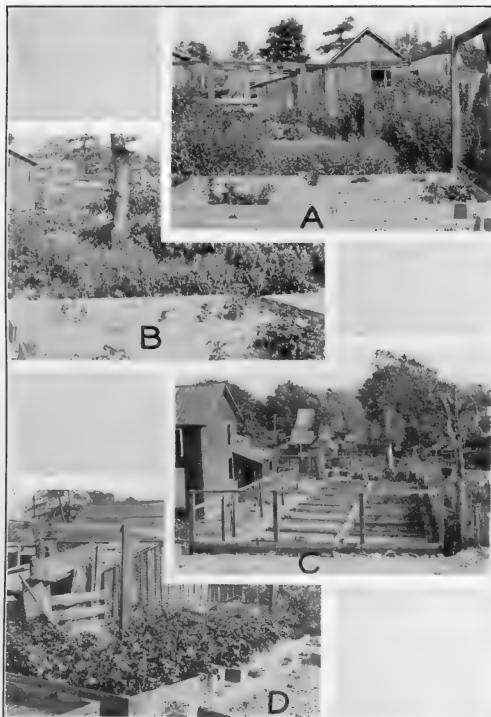
In 1919 Kunkel and Broadbent⁶ tested formaldehyde on 5 by 17 feet plots, all applications being made at the rate of 1.08 pints of solution per square foot, the strength of solution varying from 0.41 to 3.28 per cent of commercial formaldehyde. One-half of the plots were treated with cold solutions to compare with duplicate plots treated with hot (97° C.) solutions. All the plots were planted to wart-susceptible potatoes. Wart was found in each of these plots at digging time. In addition, tests were made with 10-inch earthenware pots. In these experiments the following treatments were effective in eliminating wart: Autoclaving pots filled with infected soil for 15 minutes at 15 pounds pressure; bichloride of mercury, 1 to 20,000 or stronger, applied at the rate of 40 c. c. per pot ($\frac{1}{3}$ cubic foot of soil). The following treatments failed to eliminate wart: Copper sulphate, 1 to 1,000 or weaker, applied at the rate of 40 c. c. per pot; sulphuric acid (concentrated commercial) 1 to 300, at the rate of 625 c. c. per pot; copper sulphate crystals, unweighed amounts (some pots received four uncrushed crystals of copper sulphate, averaging nearly 0.5 inch in diameter and others an approximately equal amount of crushed crystals).

CHEMICAL TREATMENTS, SPRING OF 1920

In the spring of 1920, experiments were planned to test further the results of Kunkel and Broadbent as well as to try a number of new treatments. Because of the scarcity of water in much of the wart-infected area, this factor must be taken into account in chemical treatments which require an extensive use of water solutions. The liquid treatments in the spring of 1920 were therefore planned with 2 quarts per square foot as a maximum application. The plots were 6 by 12 feet in size and located in the garden in which wart was first reported and in nearby wart-infected gardens. The plots were protected from contamination by trenching and boarding up as in the steam-pan garden, but no cages were put over the individual plots. A wire-netting fence surrounded the entire group of plots in each case (pl. 1, C).

During the summer a special study of the penetration of chemicals into the soil was begun. Chemical tests of the soil from various depths in the treated areas indicated that although an application of two quarts of formaldehyde solution per square foot might in some instances penetrate the soil to a depth of 8 inches it could not be depended upon to reach a depth of more than 4 or 5 inches. The

⁶ From unpublished manuscript and data.



A.—Potatoes in a plot treated with calcium chloride, $\frac{1}{2}$ pound per square foot, before planting time.
 B.—Potatoes in a plot treated with 1 per cent $HgCl_2$, 1 gallon per square foot, before planting time. The plot in the foreground was treated with formaldehyde.
 C.—General view of plots 1 to 20 before treatment in 1921, showing trenches, frames, and fence used to reduce possibility of reinfection by surface water or animals.
 D.—Potatoes in plots treated with Bordeaux mixture before planting time. Plot on left treated with Bordeaux 16-16-50, 1 quart per square foot, plot on right with Bordeaux 8-8-50, 2 quarts per square foot. Small parts of barren plots treated with copper sulphate and sodium fluoride respectively shown in the foreground.

failure of water solutions of chemicals in such amounts was therefore not cause for surprise. Like the plots in the steam-pan treatments, most of the chemical plots were planted to an immune variety in 1920 because of a mixture of seed, so that the results of the treatments were not obtained until 1921. These spring treatments in 1920, all of which failed to eliminate wart, included: Formaldehyde, applied so as to furnish all possible combinations of 1 and 2 per cent solutions, used hot and cold, at the rate of 1 and 2 quarts per square foot; bichloride of mercury, from $\frac{1}{2}$ pint per square foot of 1 to 32,000 to 2 quarts of 1 to 250; chloride of lime, $\frac{1}{2}$ pint per square foot of solutions made by dissolving 12 ounces or 24 ounces in $4\frac{1}{2}$ gallons of water; a commercial "potato powder" (compounded from white arsenic, lime, and copper sulphate), 1 pound to 3 gallons of water and 2 pounds to 3 gallons, $\frac{2}{3}$ pint per square foot; and Bordeaux mixture, 8-8-50 and 16-16-50, $\frac{2}{3}$ pint per square foot.

CHEMICAL TREATMENTS, FALL OF 1920

Notwithstanding the fact that the penetration tests indicated that applications of 2 quarts per square foot would immediately penetrate only 4 to 5 inches, treatments made in the fall of 1920 were confined to applications of 1 pint or less per square foot or to dry chemicals. This was done because of the difficulty that would undoubtedly be experienced in obtaining sufficient water if heavy applications were attempted on a large scale. Part of these treatments were made during rain and snow storms with the hope that the rains would carry the chemicals into the soil to a sufficient depth to insure complete sterilization. While rainfall can undoubtedly take the place of some of the water that would otherwise be required, it has not been determined to what extent this is possible. Where rainfall is reasonably heavy it is a potential source of most of the water required, as 1 inch of rain is approximately equal to 5 pints per square foot.

The plots treated with chemicals in the fall of 1920 were spaded up and planted to wart-susceptible potatoes in the spring of 1921. The results are tabulated in Table II. As shown in this table, two plots were treated with a lime-sulphur solution at the rate of 1 pint per square foot, consisting of one part of commercial solution, testing 33° Baumé, to two parts of water, and permitted very little growth. The few stunted plants that appeared bore no tubers and showed no sign of wart. No wart occurred in plots treated with crude carbolic acid, or with denatured alcohol at the rate of $\frac{1}{10}$ gallon per square foot. A plot treated with kerosene at the rate of 1 pint per square foot gave striking results. The odor of kerosene was quite evident when this plot was dug nearly a year after treatment, and the tubers were absolutely free from wart, scab, and black scurf. The plot adjoining it on the upper side had 47 per cent of wart and the tubers in nearly all plots were more or less severely infected with scab and scurf. While the plot was evidently at the edge of the infected area and may have contained relatively few wart spores, the results were such as to warrant further tests of kerosene. The plants in plots treated with creosote, potassium permanganate, sugar, and sulphur were free from wart at harvest, but the soil in them was probably only lightly, if at all, infected. Plots treated with bichloride of mercury and salt (both dry and in solution), with Bordeaux, and with chloride of lime, showed a large percentage of wart except for one Bordeaux plot in lightly infected soil. This garden was said to have

been unused for 20 years prior to 1918. Potatoes planted in it that year developed wart, although the seed is said to have been clean. The source and distribution of the disease in this garden were therefore unknown, but evidently infection was not general. No further test plantings were made.

TABLE II.—Results of chemical treatments made in the fall of 1920 on plots planted to wart-susceptible potatoes in 1921

Wart present at harvest (per cent)	Chemical used	Application		Plot ^a No.
		Formula	Rate per square foot	
0	Alcohol.....	70 per cent.....	1 pint.....	14
27	Mercuric chloride+sodium chloride.....	1 to 4½.....	9½ grams.....	10
93 to 100	Mercuric chloride+sodium chloride+water.	400 grams, 4 pounds to 25 gallons.	1 pint.....	7, 8
0 to 100	Bordeaux.....	16-16-50 or 8-8-50.....	do.....	5, 6, 16
0	Carbolic acid (crude).....	¼ gallon.....	3
47 to 48	Chloride of lime.....	50-50.....	1 pint.....	11, 12
^b 0	Creosote.....	½ gallon.....	22
0	Kerosene.....	1 pint.....	13, 15
0	Lime-sulphur.....	1 to 3, 1 to 6, 1 to 12.....	do.....	1, 2, 19, 21
^b 0	Potassium permanganate.....	1 to 25.....	do.....	20
^b 0	Sugar.....	2 ounces.....	24
^b 0	Sulphur.....	¼ or ⅛ pound.....	17, 18, 23
37 to 90	Checks.....	4, 9

^a Plots were 5 by 10 feet.

^b Soil probably very lightly infected, if at all, in these plots.

East													
North	2	4	6	8	10	12	14	16	18	20	22	24	South
	1	3	5	7	9	11	13	15	17	19	21	23	
West													

CHEMICAL TREATMENTS IN 1921

PLOT EXPERIMENTS

In the spring of 1921 four gardens with space for 100 plots, each 5 by 10 feet, were secured at Sandy Run, near Freeland, Pa., for a more comprehensive test of chemical soil sterilization. Since the preliminary tests of chemical penetration indicated that heavy applications were necessary to secure penetration, and since no dependence could be placed on rainfall in the spring, these treatments were planned for heavy applications of solution or for dry chemicals. Many of the treatments planned were so drastic as to make it almost certain that they would prevent the growth of crops for a time. It was hoped that an effective treatment could be found that would not prove injurious to crops, but wart extermination was necessarily the primary consideration. Since no effective chemical soil-sterilization treatment for this disease was known, any successful treatment, however drastic or expensive, would be a considerable advance and offer a basis for further study.

RESULTS IN 1921, 1922, AND 1923.—The details of the chemical soil treatments tested in 1921, including the strength, amount, form, and method of application, together with the results secured in wart control, are given in Table III. The diagram on Figure 3 shows the relationship of the plots to each other and shows the occurrence of wart in plots and checks in 1921 and 1922.

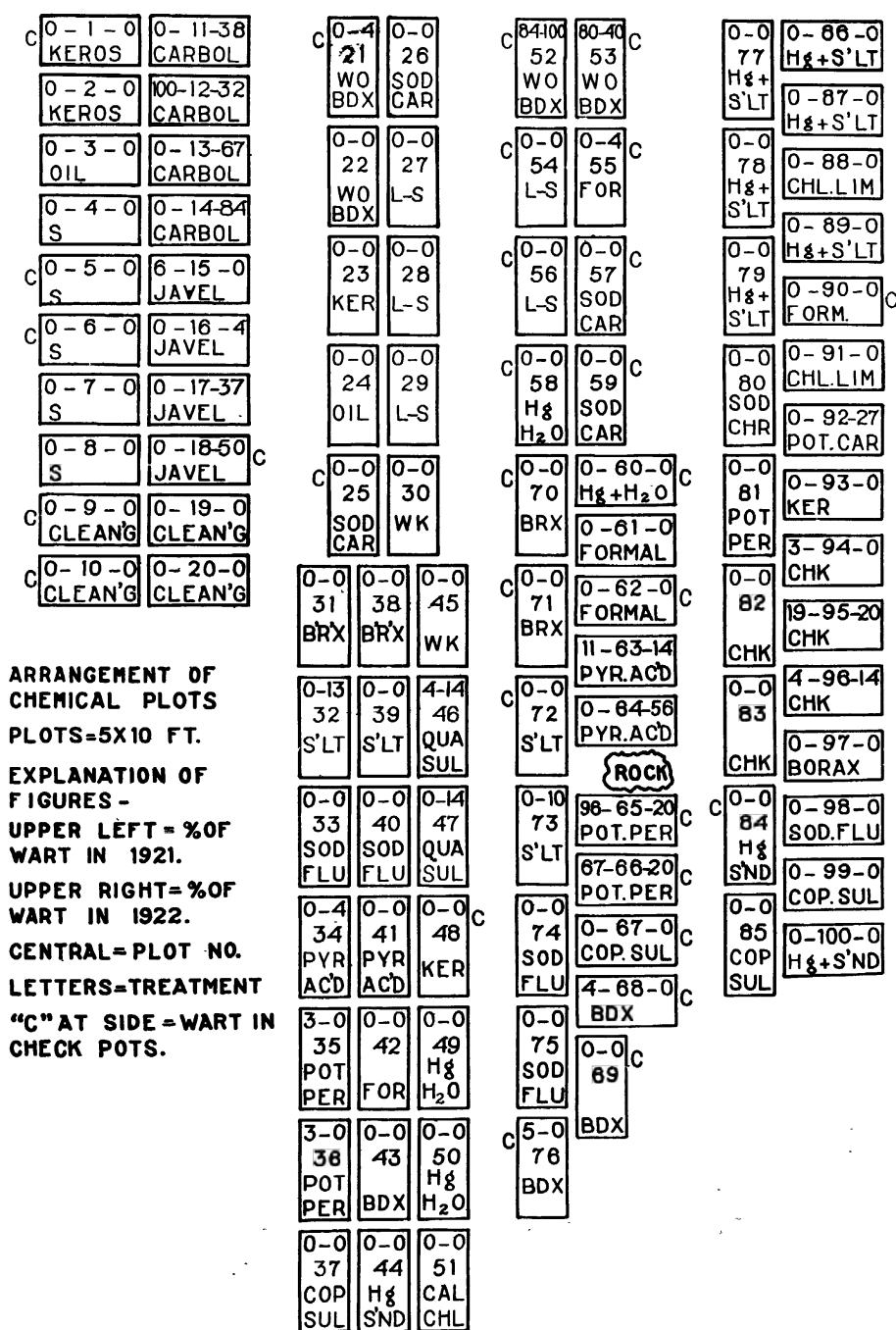


FIG. 3.—Diagrammatic drawing showing arrangement of plots in each of the four gardens used, together with the chemicals used and the results for two years. The various chemicals are indicated as follows:

Bichloride of mercury, Hg; Borax, Brx; Bordeaux mixture, Bdx; Woburn Bordeaux, Wo Bdx; Carbolic acid, Carbol; Chloride of lime, Cal Chl or Chl lim; Cleaning solution, Clean'g; Copper sulphate, Cop Sul; Formaldehyde, For, Form, or Formal; Javelle water, Javel; Kerosene, Ker or Keros; Lime-sulphur, L-S; Crude oil, oil; Potassium carbonate, Pot Carb; Potassium permanganate, Pot Per; Pyroligneous acid, Pyr acd; Qua-Sul, Qua Sul; Sodium carbonate, Sod car; Sodium chloride, Slt; Sodium chromate, Sod Chr; Sodium fluoride, Sod Flu; Sulphur, S; commercial weed killer, W K.

Sand, sand and water, H₂O, were used with bichloride of mercury in some plots. Chk=Check plot. For formulae and applications see Table III, p. 314. Before the plots were treated, two pots were filled with composite soil from each. C at the side of a plot indicates that wart was produced on the potato plant grown in one or both check pots for that plot. For a discussion of the value of these check pots see p. 316 and Table IV.

TABLE III.—Control of potato wart during three successive years, following chemical treatments applied in 1921

Per cent of hills showing wart			Exposure of plot to wart	Cost per acre	Chemicals used	Formula	Amount used per square foot	Plot No.
1921	1922	1923						
0	0	0	1 foot, C, 1 foot	\$1,900	Mercuric chloride+water	2 pounds to 25 gallons	1/2 gallon	50, 58, 81
0	0	0	1 foot, C		do	do	1 gallon	49, 60
0	0	0	1 foot		Mercuric chloride+sand	1,000 grams to 12 1/2 pounds	20 grams mercuric chloride	44
0	0	0	C		do	8 pounds to 50 pounds	do	84
0	0	0	6 feet		Mercuric chloride+sodium chloride +water	2 pounds to 50 pounds	do	100
0	0	0	6 feet, 1 foot		do	2 to 50 to 50	1 gallon	78, 89
0	0	0	1 foot, 12 feet		do	4 to 25 to 50	1/2 gallon	79, 87
0	0	0	15 feet, 18 feet		do	do	1 gallon	77, 86
0	0	0, 83, 0	1 foot, C, 1 foot		Borax	As it came	1/2 pound	38, 71, 97
0	0	0, 9 1/2	1 foot, C		do	do	1 pound	31, 70
5	0	0	C		do	8 to 8 to 50	1/4 gallon	76
4	0	0	do		Bordeaux (regular)	16 to 16 to 50	do	68
0	0	0	1 foot, C		do	8 to 8 to 50	1/2 gallon	43, 69
0	0	4, 0	C, 1 foot	315	Bordeaux (Woburn)	6 to 6 to 100	1 gallon	21, 22
84, 80	100, 40	100, 78	C, C		do	do	2 gallons	52, 53
100, 0	32, 84	13, 14	1 foot, 1 foot		Carbolic acid crystals	5 per cent	1/2 gallon	12, 14
0	38, 67	12 1/2, 4	do		do	do	1 gallon	11, 13
0	0	32, 0	6 feet, 6 feet		Chloride of lime	As it came	1/2 pound	51, 88
0	0	0	1 foot	2, 100	do	do	1.6 pounds	91
0	0, 0, 0	0	C, C, 1 foot	7, 550	Cleaning solution	do	1 gallon	9, 10, 19
0	0, 0, 0	0	1 foot		do	Crushed crystals	2 gallons	20
0	0, 0	0	1 foot, 1 foot	1, 750	Copper sulphate	5 per cent	1/2 pound	37, 85
0	0, 0	0	C, 1 foot		do	10 per cent	1 pound	67, 99
0	0	0	1 foot, 1 foot		Formaldehyde, commercial	15 per cent	2 gallons	42, 61
0	0	14, 4	C, C		do	40 to 10 to 25	do	55, 62
0	4, 0	0	1 foot, 1 foot	24, 400	Javelle water	3/4 to 1/4 to 25	1 gallon	90
6, 0	0, 4	0	1 foot, 1 foot		do	As it came	do	15, 16
0	37, 50	12, 14	1 foot, C		Kerosene	do	1/2 gallon	17, 18
0	0	0	1 foot	4, 900	do	do	1 gallon	93
0	0	0	C, 1 foot		do	do	2 gallons	1, 2
0	0	0	6 feet		Kerosene and crude oil	do	do	48
0	0	0	1 foot	728	Lime-sulphur	1 gallon of each	do	23
0	0	0	6 feet, C		do	1 in 12	1 gallon	29
0	0	0	1 foot, C		do	1 in 6	1/2 gallon	28, 54
0	0	0	1 foot, C		do	1 in 3	do	27, 56
0	0	0	6 feet, 1 foot	10, 900	Oil, crude	As it came	1 gallon	3, 24
0	27	58	1 foot, 1 foot		Potassium carbonate	do	1/2 pound	36, 92
3, 96	0, 20	4, 59	1 foot, C		Potassium permanganate	1 to 1,000	1 gallon	35, 66
3, 67	0, 20	0, 9	do		do	do	2 gallons	

0, 11	0, 14	12½, 9	1 foot, 1 foot.	Pyroligneous acid	As it came.	1½ gallon.	41, 63
b 0	4, 56	4½, 74	do.	do.	do.	¾ gallon.	34, 64
4	14	4	1 foot.	Qua-Sul	1 to 50.	1½ gallon.	46
b 0	b 0	0	do.	do.	1 to 100.	1 gallon.	47
b 0	b 0	0	O, C.	Sodium carbonate	As it came.	2 pounds.	25, 57
b 0	b 0	0	1 foot, C.	do.	do.	3 pounds.	26, 59
b 0	0, 10	14, 32	1 foot, 1 foot.	Sodium chloride	do.	1½ pound.	39, 73
b 0	13, b 0	12½, 100	1 foot, C.	do.	do.	1 pound.	32, 72
b 0	b 0	0	do.	Sodium chromate	1 pound to 2 gallons	2 gallons.	80
b 0	b 0	b 0	do.	Sodium fluoride	As it came.	¾ pound.	98
b 0	b 0	b 0	1 foot, 1 foot.	do.	do.	1½ pound.	40, 75
b 0	b 0	b 0	do.	do.	do.	1 pound.	33, 74
b 0	b 0	0	C, 1 foot.	Sulphur	do.	1½ pound.	6, 8
0	b 0	b 0	do.	do.	do.	¾ pound.	5, 7
0	b 0	0	1 foot.	Sulphur + inoculated	do.	1½ pound.	4
b 0	b 0	0	do.	Weed killer /	10 per cent.	1 gallon.	30
b 0	b 0	b 0	do.	do.	20 per cent.	do.	45
0	0	4, 14	1 foot, 1 foot.	None	do.	do.	82, 83
3, 19, 4	0, 20, 14	31, 65, 14	1 foot, 1 foot, 1 foot.	do.	do.	do.	94, 95, 96

Plots were 5 by 10 feet. Dry chemicals were raked or spaded in.

a Cost per acre as given is cost of materials only for the minimum successful application.

b Growth poor or none. Growth in plots receiving the same treatment often varied.

c C.—Wart occurred in one or both of the pots filled with soil from this plot.

d Plot 81 was treated with potassium permanganate, 2 ounces to 10 gallons, 1½ gallon per square foot, previous to its treatment with mercuric chloride.

e The inoculated sulphur used was furnished free of charge by W. H. Martin of the New Jersey Agricultural Experiment Station.

f A solution of arsenite of soda, sodium chloride, and sodium carbonate.

Where this is indicated the notations are for plots in the same order as plot numbers in the right hand column.

1 foot.—Wart occurred in a plot 1 foot away or in one or both of its check pots. Exposures are in the same order as the plot numbers in the last column.

As shown in Table III, plots receiving treatments by 14 chemicals remained wart-free through the three growing seasons 1921, 1922, and 1923. These chemicals were bichloride of mercury, Bordeaux mixture, chloride of lime, cleaning solution, copper sulphate, formaldehyde, kerosene, lime-sulphur, crude oil, sodium carbonate, sodium chromate, sodium fluoride, sulphur, and a commercial weed killer.

Many of the plots treated with these chemicals showed very little growth ⁷ of potatoes during the first season, 1921, some showed poor growth in 1922, and a few showed poor development in 1923. In the plots treated with sodium fluoride growth was entirely prevented during all three seasons.

The gardens used for these experiments were reported to be heavily infected with *Synchytrium endobioticum*. Soil samples from different parts of each garden were prepared in 50 per cent sugar solutions and centrifuged in order to secure visual evidence of the presence of wart sporangia. As a further check, designed to show whether or not the sporangia present were viable, two 10-inch pots were filled with composite soil from each plot before the plot was treated, and potatoes were planted in these pots the first year (1921). A "C" is shown in Figure 3, beside a number of plots indicating that wart developed in one or both of the pots filled with soil from that plot. Previous experience with pot checks had indicated definitely that they could not be depended on to give an accurate indication of the presence of wart in the plot soil. The following tabulation (Table IV) of the occurrence of wart in the check pots in 1921 as compared to the occurrence of wart in the corresponding plots brings out this point.

TABLE IV.—Check pots as indicators of the presence of wart in plots

Gardens containing plots	Wart present in check pots		Wart not present in check pots	
	Wart in plots	No wart in plots	Wart in plots	No wart in plots
1 to 20.....	1	5	7	7
21 to 51.....	1	2	9	19
52 to 76.....	11	8	3	3
77 to 100.....	0	2	* 6	16
	13	17	* 25	45

* Five of these plots were untreated

The pots were planted in 1921, only, but the plots were planted in 1921, 1922, and 1923; and no distinction is made between plots producing wart one year, two years, and three years.

It will be noted that the check pots demonstrated the presence of viable wart spores in 30 plots and only 13 of these plots produced wart. However, 16 of the remaining 17 plots received treatments which prevented wart throughout the experiments. No wart developed in either of the check pots for 70 plots, yet wart developed during one or more years in 25 of these plots. The pots demonstrated the presence of wart spores in only 30 plots, whereas they were present in at least 55 plots.

⁷ The growth obtained is discussed in the section on Potato Growth Following Certain Chemical Soil Treatments (Part IV).

It is interesting to note that no wart developed in the check pots for the 5 untreated plots. Only 2 of these 5 plots produced wart all three years. Another produced wart the first and third years. Two produced wart the third year only. While these untreated plots were in the garden showing the least wart, the results obtained serve to emphasize the necessity of continued planting of treated plots in order to make sure that the results obtained are dependable.

Treatments that are sufficiently drastic to prevent or greatly retard the growth of potato plants might be expected to kill any swarm spores produced by the wart organism as long as the inhibiting factor remains. Unless the highly resistant resting sporangia are killed or die in the meantime, it is conceivable that they might cause wart after the factor which inhibits plant growth is eliminated from the soil. Plots which showed no wart during the three growing seasons above mentioned may be grouped according to the adequacy of growth of potatoes in them to give a reliable test of the presence of viable wart spores, as follows: Plot showing no growth, that treated with sodium fluoride; plots showing inadequate growth, those treated with sulphur; plots showing doubtfully adequate growth, those treated with copper sulphate crystals, lime-sulphur solution, sodium carbonate, sodium chromate, a commercial weed killer; plots showing probably adequate growth, those treated with bichloride of mercury (in part), chloride of lime, cleaning solution, formaldehyde, kerosene; plots showing good growth, apparently adequate for a good test, those treated with Bordeaux mixture, bichloride of mercury (in part), crude oil.

RESULTS OF POT EXPERIMENTS WITH BICHLORIDE OF MERCURY AND SODIUM CHLORIDE

The efficiency of bichloride of mercury and salt solutions for killing the wart organism in the soil was tested by using treated wart-infected soil to inoculate potatoes. Two-inch glass soil tubes⁸ were filled with thoroughly infected soil from one of the gardens in Free-land, Pa., the soil was compacted, a 1 per cent solution of mercuric chloride plus 5 per cent sodium chloride was made and applications added at the rate of 2 quarts per square foot. This treatment was followed almost immediately by water at the rate of 2 quarts per square foot. Chemical tests on part of the tubes showed the presence of mercury to a depth of 8 to 9 inches in the soil. At the end of three weeks, pots were partly filled with wart-free soil and potato seed pieces placed on top. Then soil was removed from the treated tubes, and from checks, an inch at a time, and sprinkled over the seed pieces and soil in the pots. The pots were then filled with wart-free soil. The potatoes made normal growth and at the end of three months were examined for the presence of wart. The 90 pots inoculated with soil from the surface to the 9-inch depth of treated tubes showed no wart. In 6 of the 29 pots inoculated with soil from below the 9-inch depth of treated tubes wart developed. In 10 of the 35 pots inoculated with untreated wart-infected soil from check tubes wart developed. A duplicate set of tubes gave almost exactly the same amounts of wart. So little of the treated soil was used in each pot

⁸ These tubes were used in the study of the penetration of soil fungicides and are described on p. 331. The methods used are discussed on pp. 330-334.

that if the wart spores were not killed, any inhibitory action of the chemicals should have been overcome and the disease produced in at least some of the pots.

SUN AND AIR TREATMENTS

In planning soil treatments with a view to the extermination of the potato wart it is necessary to know something of the rate and methods of spread of the pathogen in order to know how far beyond the known limits of the infected area soil treatment will be necessary. It seems certain that wart spores are present in wind-borne soil, but the slowness with which the wart disease seems to spread in infected gardens indicates that comparatively few infections result from wind-borne soil. It was thought that relatively short exposure of spores to air and sun might be sufficient to kill them. An experiment to test this was carried out in the greenhouse during the winter of 1920-21. Wart-infected soil was spread in thin layers, $\frac{1}{8}$ and $\frac{1}{4}$ inch deep, on wart-free soil in pots in the greenhouse and exposed to air and sunlight for from 2 to 12 days. The wart-infected soil was used as it came from the garden except that where necessary lumps were broken and stones and débris removed in order that the total thickness of the layers of soil should not exceed the specified $\frac{1}{8}$ or $\frac{1}{4}$ inch. At the end of the exposure periods the pots were planted to potatoes by pressing a single tuber partly into the soil in each pot and covering it with wart-free soil. Of the 48 tubers planted in pots containing $\frac{1}{8}$ inch of this wart-infected soil, 44 produced wart-infected plants. Of the 48 tubers planted in pots containing $\frac{1}{4}$ inch of wart-infected soil, 36 produced wart-infected plants. Of the 20 tubers planted in pots containing $\frac{1}{8}$ or $\frac{1}{4}$ inch of the same lot of wart-infected soil and not exposed to air and sun, 8 produced wart-infected plants. These results seem to indicate that wart spores near the surface of freshly cultivated soil retain their vitality for some time, long enough for the soil to dry out and be blown about.

In another experiment 60 tubers were wet, rolled in wart-infected soil from the same source and exposed to air and sunlight for from 2 to 25 days. Only 3 of these tubers, exposed 2, 9, and 12 days, developed wart when planted. The fact that nearly all of the soil had fallen from these tubers before they were planted may account at least in part for the low percentage of infection obtained.

COST OF TREATMENTS

The cost of many of these treatments is much too high to warrant their use except under special circumstances.

COST OF STEAM-PAN TREATMENTS

With a minimum of overlapping a 6 by 12 foot steam pan would treat 65 square feet of soil at a time, making 670 pan areas per acre. Steaming 1 pan area every $1\frac{1}{2}$ hours would accomplish the treatment of 5 pan areas each 8-hour day. The cost would approximate \$15 per day divided as follows: Engineer \$5, 2 laborers at \$4, one-third ton of coal at \$6 per ton; or \$2,010 per acre. In addition there would be the cost of water, which would have to be hauled or pumped; drayage on coal; transportation of men; rental or depreciation of outfit; probability of higher labor costs, especially for engineers;

cost of a trained supervisor. The foregoing costs assume that the laborers could do all razing and replacing of fences and poles and all treatments of soil that could not be treated by the steam pan. In this work a 5 by 9 foot and a 6 by 9 foot steam pan were used, which weighed about 400 pounds each; but with several strong men or special devices, larger ones could be used. Treatment would obviously be so slow that several outfits could probably be used to advantage, greatly reducing the cost of transportation of labor and coal and possibly enabling a small crew to handle all labor for several outfits. It seems doubtful if the cost could be reduced much, if any, below \$1,500 per acre.

COST OF CHEMICAL TREATMENTS

The applications of chemicals made in 1921 were extremely heavy, and it seems probable that positive results have been obtained with such of these chemicals as are capable of effective sterilization. The quantity of chemical used for some of the treatments could doubtless be reduced considerably without loss of efficiency. It has not been possible to carry out experiments to determine the most effective and economic quantity of each chemical used. None of the treatments have been tried on a sufficiently large scale to make possible the giving of estimates of the cost per acre of application of the materials listed. The steam used in the steam-pan experiments was furnished free of charge by a local coal company. Treatments requiring water would be relatively expensive to apply since there would be large quantities of solution to handle, and water solutions soak into the ground more or less slowly. In much of the region where potato wart occurs, the water supply is inadequate during most of the growing season and special reservoirs would have to be constructed to retain the spring run-off if water solutions were to be used on a large scale. Experiments have shown that Bordeaux mixture will penetrate the soil hardly at all and must be worked in. Kerosene and crude oil penetrate so quickly that their application would only require an accurate sprinkler or distribution system. The application of dry chemicals should be as cheap and easy as the application of fertilizers.

The prices of some of the chemicals used are extremely variable, sometimes as much as 50 per cent variation from one year to the next. Unless otherwise specified, the prices given below are those quoted in the General Schedule of Supplies of the United States Treasury Department, 1924.

Cost of materials per acre for chemical treatments preventing wart

Bichloride of mercury.....	\$1, 896. 06
\$5.75 for 2,500 grams. One-half gallon of 1 per cent solution per square foot. With 5 per cent of salt added to give better and more even distribution, the cost would be increased about \$83.60 per acre, salt being 92 cents per hundredweight.	
Bordeaux mixture.....	313. 55
Copper sulphate 8 cents per pound, lime (estimated) 1 cent per pound 8-8-50, ½ gallon per square foot.	
Chloride of lime.....	2, 090. 88
3 cents per pound, 1.6 pounds per square foot.	
Cleaning solution.....	7, 550. 40
Sodium chromate, 15 cents per pound. Sulphuric acid (sp. g. 1.84), 36.8 cents per gallon (5.28 cents per kilo) 10 pounds sodium chromate, 3 gallons sulphuric acid, 12 gallons water, 1 gallon per square foot.	

Copper sulphate.....	\$1, 742. 40
8 cents per pound, $\frac{1}{2}$ pound per square foot.	
Formaldehyde.....	24, 441. 44
\$29.60 for 60 kilos. Two gallons of 15 per cent solution per square foot.	
Kerosene.....	4, 878. 72
14 cents per gallon (price paid in 1921), $\frac{1}{2}$ gallon per square foot. Results with 1 pint per square foot were very promising, so that application could probably be greatly reduced; cost at 1 pint per square foot would be \$762.30.	
Kerosene and crude oil.....	16, 988. 40
Kerosene 14 cents, crude oil 25 cents per gallon (prices paid in 1921). One gallon of each per square foot. The "crude oil" looked like a good grade of machine oil. Crude oil can sometimes be obtained at oil wells at 5 cents per gallon.	
Lime-sulphur.....	726. 00
Concentrated lime-sulphur solution 20 cents per gallon (estimated) 1 part in 6 parts solution, $\frac{1}{2}$ gallon per square foot.	
Oil, crude.....	10, 890. 00
25 cents per gallon (price paid in 1921) (see above under kerosene and crude oil). 1 gallon per square foot.	
Sodium carbonate.....	1, 411. 34
1.62 cents per pound, 2 pounds per square foot.	
Sodium fluoride.....	3, 136. 32
18 cents per pound, $\frac{2}{5}$ pound per square foot.	
Sulphur.....	217. 80
4 cents per pound (estimated), 2 ounces per square foot.	
Weed killer.....	6, 534. 00
Weed killer, \$1.50 per gallon (price paid in 1921) 1 gallon of 10 per cent solution per square foot.	

The cost of materials in the foregoing list is based on the amounts actually used. Only a few of these treatments, bichloride of mercury, Bordeaux mixture, and copper sulphate, were tried in smaller quantities, but with resulting failure. It seems probable that at least some of the quantities used could be markedly reduced without prejudice to the results. Sodium fluoride at the rate used, 20 pounds per plot of 50 square feet, absolutely prevented growth during all three years. It would seem likely that a considerable reduction could be made in the rate of application of this chemical. The amount of chemical used per square foot could very likely be reduced for cleaning solution, crude oil, sodium carbonate, and weed killer. Cleaning solution is known to be a strong, rapid killing agent and in the amount used made a thin mud of the soil to the depth spaded up. Crude oil of the type used penetrates soil very readily, and sufficient depth of penetration could probably be obtained in most soils with smaller applications. Applications of sodium carbonate were very heavy, 2 pounds per square foot, and prevented growth during the first two years. Weed killer at the strengths used prevented the growth of potatoes during the first two years. It is designed to prevent the growth of green plants, and its action on wart sporangia might of course be only a temporary inhibition. Kerosene used as a fall treatment at 1 pint per square foot gave good results, but needs further testing.

SUMMARY

The only way to eradicate potato wart in infected soil quickly and effectively is by soil sterilization.

Soil may be sterilized by heat or by chemicals.

The inverted steam-pan method is the most approved method of heat sterilization of soil.

Although effective, steam-pan sterilization is slow, cumbersome, and expensive.

The application of small amounts of formaldehyde or of bichloride of mercury solutions just previous to steaming the soil can not be depended on to increase the efficiency of the treatment.

Of the 22 chemicals tried in the dilutions and amounts given, 14 produced wart-free plots during the season treated and during the two following seasons.

Six of the successful chemicals (bichloride of mercury, chloride of lime, copper sulphate, sodium carbonate, sodium fluoride, and sulphur) were used dry. Kerosene and crude oil were used undiluted. Water was used with seven of the chemicals (bichloride of mercury, Bordeaux mixture, cleaning solution, formaldehyde, lime-sulphur, sodium chromate, and a commercial weed killer).

Water is difficult to obtain in the principal region infected by potato wart, at Freeland, Pa., and this adds to the cost of applying chemicals in solution. The use of chemicals dry or applied as strong solutions during the late fall would be desirable if the amount of water required could thereby be reduced. Such data as were obtained on this point indicate that such a reduction might be possible.

Some test plots gave such poor growth of potatoes that conditions were not favorable to wart infection, and final conclusions as to the effectiveness of the chemicals used on them were therefore not possible. Sodium fluoride prevented growth all three seasons. Sulphur nearly prevented growth all three seasons. The growth in plots treated with copper sulphate crystals, lime-sulphur, sodium carbonate, sodium chromate and a commercial weed killer was such that the test of their efficacy is not considered entirely satisfactory. The growth in at least part of the plots treated with bichloride of mercury, cleaning solution, kerosene, Bordeaux mixture, and crude oil was such as to make it seem almost certain that wart would have developed if the treatment had not been effective.

The approximate cost per acre for the materials used in the successful treatments is given. Some of the more promising treatments requiring no water are sulphur, \$220 per acre, and kerosene, \$765 per acre. Two promising treatments requiring water are lime-sulphur, \$726 per acre, and Bordeaux mixture, \$315 per acre. Bichloride of mercury in various combinations seems undoubtedly effective but costs \$1,900 per acre for materials. Steam-pan treatments if sufficiently prolonged would be effective where usable but would probably cost at least \$1,500 per acre.

PART II.—TEMPERATURE CHANGES OCCURRING IN SOIL UNDER A STEAM PAN

INTRODUCTION

In connection with the test of steam-pan treatments as a possible means of exterminating the potato-wart organism in the United States, data were secured as to the temperature changes occurring in the soil under the steam pan. If a given amount of steam heat is fatal to the potato-wart organism, a knowledge of the conditions necessary to insure that amount of heat in each type of soil to be treated and to the proper depth is essential to efficiency in using the treatment on a large scale.

Temperatures obtained in soil treated by the steam-pan method have apparently received relatively little attention. A number of investigators, including Russell and his associates (15, 16, 17, 18) in England, and Johnson (10, 11) in this country, have studied the effect of heat on soil and on plant growth, but no schedule of steam-pan temperatures was found in the literature. So far as the writers have been able to discover, the most complete schedule of temperatures obtained under a steam pan are given by Beinhart (2), who says, "In sandy soils, after 30 minutes of steaming, the temperatures to be expected in the upper two inches of soil directly under the pan are approximately 208° to 212° F., at 3 to 4 inches 170° to 180°, and at 6 inches 120°. Two hours after the removal of the pan the temperature at 6 inches should be about 160° F."

The work herewith reported gives a much more comprehensive series of temperatures than those of Beinhart.

APPARATUS AND METHODS

The construction of the steam pans used in the potato-wart extermination experiments is explained on pages 303 and 304.

The source of steam was a high-pressure 8-inch main which carried steam for use in the coal mines for pumping, hoisting, and other purposes. The pressure in the main was almost constant, usually varying only between 135 and 140 pounds, but often dropped appreciably for a few moments, particularly when hoisting outfits were being started. The quality of the steam varied considerably. The point at which the steam-pan supply line was attached to the main was said to be several miles from the boiler generating the steam. When rain fell on the exposed part of the pipe line there was more condensation than usual. The steam was also much poorer, i. e., contained more condensation water, when the mines were using little steam.

During the early part of the season the steam pan was located 200 feet from the large main, $\frac{3}{4}$ -inch pipe being used to carry the steam almost to the pan, 20 feet of steam hose being used at the pan. Later in the season the pan was used only 75 feet from the steam line. Steam at 140 pounds pressure is not readily managed and is not always available, hence it was thought best to reduce the pressure. Beinhart and others recommended the use of steam at 100 pounds boiler pressure with a minimum of 70 pounds. In the writers' work a gauge was placed on the pipe line near the point of attachment of the steam hose and only enough steam turned on the closed line to give a reading of 90 to 95 pounds on this gauge. This method of regulating the steam was not entirely satisfactory when the steam pan was 200 feet from the main. When the pan was moved to 75 feet from the main it was found practically impossible to have the gauge pressure at 90 to 95 pounds with the line closed and then have any steam flow at all when the steam was turned on the pan. It was found desirable to take the gauge reading with the steam running wide open into the air. Under these conditions a gauge reading of between 5 and 10 pounds, nearly 10 pounds, seemed to give a flow of steam equal to that formerly obtained with the 95 pounds pressure. The observations made led to the conclusion that the flow of steam was probably different following each separate setting of the valve by gauge reading with the line closed, even when the pan was 200 feet away. In order to secure an accurate index of the steam flow a

reducing valve or "pressure-regulator" was secured. A small gauge was put on near the end of the line of pipe and the reducing valve placed between this gauge and the steam main was set so that the gauge would read 10 pounds with the line wide open. While sudden calls on the main for hoisting, or other work in the mines sometimes made the pressure drop to 7 or 8 pounds for a moment, the steam flow was practically uniform for the entire period of steaming. By this method the steam could always be regulated in a few minutes, whereas formerly it usually took a half-hour or more. A measure of the quantity of steam flowing into the steam pan is essential for comparative purposes and to secure the uniform treatment of the different pan areas supposed to receive the same treatment. Where the pressure on the main or boiler varies, or where the outlet must be regulated to keep the boiler pressure up, even an approximate estimate of the steam flow is difficult. In such cases the use of an inexpensive reducing valve will do much to insure the delivery of uniform quantities of steam in a given time.

The gardens used in our experiments are located at the southern boundary of the glacial advance, the soil being a mixture of the DeKalb and Volusia series of silt loams. The soil in these gardens was the usual conglomeration of soil, coal dust, and débris found in the gardens of coal miners in that region. As might be expected, the soil often varied considerably in different parts of the same garden and even in different parts of the same pan area.

Electrical resistance bulbs were used to obtain the temperatures in soil under the steam pan. These resistance bulbs were connected to a Wheatstone Bridge on which the dial was graduated to show temperature in degrees Centigrade instead of showing the corresponding electrical resistance. The writers can not recommend these bulbs for this work, as they get out of order too readily when held at approximately 100° C.

Soil to be given a steam-pan treatment was spaded, and if lumpy the lumps were broken up, yielding a surface layer of well-cultivated soil 8 to 9 inches deep. When temperatures were to be taken, one or more trenches were dug in this prepared soil and the leads placed in them and covered. The depth of the soil over the leads was measured by laying a board across the trench and measuring from the lower edge of the board to the top of the cap or bulb on the end of the lead. If the depth was not that desired more soil was added or some scooped out until the bulb rested at the proper level. By carefully removing the soil after the treatment was finished and re-measuring the depth to some of the bulbs it was determined that the method of placing the bulbs was accurate. In thus putting in the leads and getting them at exactly the right level it was usually necessary to add a layer of rather fine soil. In addition, the extra handling tended to break up the soil somewhat so that the soil surrounding the leads was undoubtedly finer than the main body of soil to be treated. The soil was not compacted. The leads were put in approximately parallel to the surface, so that the channels inevitably formed where the soil was in contact with them probably had little or no effect on the results obtained. Sometimes several leads were put in the same trench at different levels. When this was done care was taken to keep the bulbs from being placed one immediately over the other, even though 2 or 3 inches of soil might intervene.

When all leads were installed the steam pan was placed on the area and preliminary temperatures were taken. The preliminary temperatures sometimes varied a degree or two, especially on hot days, even though the soil was newly spaded. The surface soil would get warmed up considerably and tend to retain its heat long enough to show when the reading was made, even if it had been used to cover one of the lower leads and had later been covered by other soil. After preliminary temperatures were recorded, steam was turned on. The steam was always allowed to run wide open into the air for a few minutes to drive out any accumulated water of condensation before the steam was turned into the steam pan. Sometimes a number of gallons of water would be blown out, sometimes practically none would come out. During the time the steam was on and for an hour afterward temperatures were usually taken every five minutes. The steam pan was almost always left on for 30 minutes after steam was shut off.

SOME VARIABLE FACTORS

The particular part of the pan area chosen for placing the bulbs did not seem to affect the results. The temperatures obtained with two bulbs at the same depth in different parts of the same pan did not always agree, but the variation was not constant for different tests.

For a great many pan areas the percentage of moisture present in the soil was determined, but these data are not now available. Such data as are available, however, and the impression gained from observations at the time of steaming fail to indicate any particular correlation between the amount of soil moisture and the penetration of heat. In most cases steaming did not seem to greatly increase the soil moisture. Six samples from pan areas steamed for 40 minutes and having moisture percentages ranging from 23 to 33 and averaging 27 before steaming ranged from 25 to 50 per cent, averaging 32 per cent of moisture after steaming. This is an average increase of 5 per cent. If one sample showing an increase of 20 per cent (30 to 50 per cent) is omitted the average increase is only 2 per cent.

The increase in the percentage of moisture may depend on the type of soil more than on the quality of the steam. Of the six samples averaged, as indicated above, three were from the same pan area. In this case the soil was prepared as usual and three ditches scooped out for leads. In the middle ditch a single lead was installed, using the soil that had been removed from the ditch to cover the bulb and lead. In the left-hand ditch three bulbs were installed, yellow claylike subsoil being used to fill the ditch. In the right-hand ditch three leads were packed in black soil containing a large amount of fine fibrous rootlets and humus. In the normal soil the moisture increased from 25 per cent before steaming to 26 per cent after steaming; in the clay soil the moisture was 33 per cent both before and after steaming; in the black soil the moisture increased from 30 to 50 per cent. The lead in the normal soil was buried to a depth of 4 inches. It showed a maximum temperature of 44° C. in 90 minutes. The 4-inch lead in yellow subsoil showed a maximum temperature of 55° in 90 minutes. The 4-inch lead in the black soil showed a maximum temperature of 61° in 65 minutes.

Soil samples for moisture determinations were taken from 3 to 5 inches below the surface in order to get soil more nearly representing

the average. The surface soil was, of course, always damp or wet when the pan was removed, but lost much of this excess moisture within a few hours.

An attempt was made to determine the temperatures in undisturbed soil by inserting the leads in horizontal holes made by driving a rod in from a trench along one side of the soil to be treated. It did not seem possible to place the leads accurately enough in this way to get results that could be averaged without running a large number of tests. The results obtained did not seem to indicate that any marked differences in temperatures would be found between the newly spaded and the unspaded soil. If this should be found to hold true under all conditions it would obviate the necessity for cultivating the soil before steaming it.

DISCUSSION OF TEMPERATURES OBTAINED

A summary of the more important data obtained is given in Table V. A considerable number of tests are not included in this summary because the leads were placed at other depths, or because the temperatures were not taken for a long enough time, or because something went wrong with the apparatus before the series of temperature readings was complete. Hence the small number of figures averaged does not necessarily measure the real value of the data. In any event the data given represent a great advance over those hitherto available.

In Table V, temperatures obtained at 1, 4, and 7 inch depths only are considered. The data from the different pressures and lengths of steaming are arranged to show the maximum temperatures obtained the number of minutes taken to reach that temperature, the temperatures recorded at different times afterward, the last temperature taken, the length of time elapsing after the maximum temperature was reached until the final temperature was taken, the rate at which the temperature increased from the initial to the maximum temperatures (given in minutes elapsing for each degree of rise in temperature), the rate at which the temperature declined from the maximum to the last temperature recorded (given in minutes elapsing for each degree of fall in temperature), and the "maximum sustained temperature" arrived at by averaging the temperatures that will give the highest average for a half-hour period (the table showing the minimum temperature used in securing this average as well as the maximum and average temperatures). The number of sets of figures averaged for each line of figures given in the table is shown in the column at the extreme right.

The table of temperatures (Table V) has been made sufficiently complete to enable anyone to make curves showing the rise and fall of temperatures for each treatment and depth considered and showing the amount of heat received by each depth, as indicated by these temperatures and the elapsed time, over a period of three or four hours. The maximum sustained temperatures and the pressures and times necessary to attain them, with which the results of treatments given may be correlated, are perhaps the most important figures for potato-wart work. The data as a whole may be of considerable value to others, particularly perhaps to those engaged in a study of the biological changes induced by steam-pan treatments of the soil.

TABLE V.—*Temperature changes in degrees centigrade occurring in soil under a steam pan, showing the temperatures 1, 4, and 7 inches below the surface in the treated soil during and following steam-pan treatments, at different pressures and for different lengths of time. Rates of rise and fall in temperatures and maximum temperatures sustained for 30 minutes are included*

95 pounds pressure, with outlet closed,* 25 minutes ^b																
Depth of soil covering temperature bulbs	Initial temperature of soil	Maximum temperature of soil	Time to attain maximum temperature	Temperatures after maximum had been reached					Average last temperature taken	Average time from maximum to last temperature	Average time required per degree of increase from initial to maximum temperature	Average time required per degree of decrease from maximum to last temperature	Maximum sustained temperatures maintained for one-half hour			Sets averaged
				10 min.	20 min.	30 min.	45 min.	60 min.					Min.	Max.	Aver.	
In.	° C.	° C.	Min.	° C.	° C.	° C.	° C.	° C.	° C.	Min.	Min.	Min.	° C.	° C.	° C.	No.
1	22	88	29	81	75	70	58	50	33	215	0.45	3.6	70	89	81	5
4	23	48	72	47	47	45	43	41	33	172	2.9	11.9	44	53	47	4
7	22	29	134	29	28	28	29	29	28	107	19.1	107	27	30	28	4
95 pounds pressure, with outlet closed, 40 minutes																
1	22	98	32	96	92	88	77	63	37	225	0.42	3.3	86	99	95	4
4	20	74	54	71	70	69	65	63	45	188	1.0	6.5	47	99	71	5
7	19	38	151	37	37	37	37	36	35	110	7.9	40	33	47	37	4
10 pounds pressure, with outlet open,* 25 minutes																
1	21	96	20	91	88	85	74	54	34	212	0.27	3.4	85	100	94	5
4	20	74	57	73	71	68	63	59	35	209	1.1	5.6	41	99	73	5
7	19	56	100	53	51	50	47	46	34	161	2.7	8	26	89	53	5
10 pounds pressure, with outlet open, 40 minutes																
1	19	99	29	98	95	90	78	63	35	209	0.36	3.3	91	99	97	13
4	17	72	62.5	68	65	63	60	57	41	187	1.1	6	38	89	68	17
7	16	38	155	38	37	37	36	32	31	131	7.0	58	23	58	37	14
10 pounds pressure, with outlet open, 75 minutes																
1	14	100	47	100	99	99	97	91	40	291	0.55	4.8	100	100	100	3
4	14	100	62	99	98	96	92	87	54	276	0.72	6	98	100	99	3
7	15	100	70	97	95	92	85	79	52	268	0.82	5.6	96	99	97	3
20 pounds pressure, with outlet open, 40 minutes																
1	14	100	15	100	99	98	97	96	39	152	0.17+	2.5	98	100	99.5	6
4	13	100	32	99	98	96	94	84	61	134	0.37-	3.4	99	100	99	9
7	13.5	100	38	98	90	85	79	75	64	119	0.44	3.4	94	98	96	6

* 95 pounds pressure with outlet closed means that a gauge on the steam line registered 95 pounds before the steam outlet was opened.
^b The number of minutes during which steam was allowed to run into the steam pan is indicated. In each case the steam pan was removed 30 minutes after steam was shut off.
^c 10 pounds pressure with outlet open means that a reducing valve was used and the valve so adjusted that a gauge on the steam line between the valve and the outlet registered 10 pounds pressure, while steam was allowed to run freely into the open air.

It has been stated that the flow of steam secured where the pressure was regulated to 85 to 95 pounds with the outlet closed appeared to be equal to that secured when the steam was regulated to about 10 pounds gauge pressure with the outlet wide open. This opinion was originally based on the appearance and sound of the flowing steam. The table shows that the temperatures obtained were similar also. The temperatures obtained with 25 minutes of steaming at 10 pounds are abnormally high for some unknown reason, the temperature at the 7-inch depth being greater than was secured at this depth with 40 minutes of steaming at the same gauge pressure. The maxima after 25 minutes of steaming show a discrepancy largely attributable to this. The maxima obtained after 40 minutes of steaming are as nearly identical as could be expected, the 1, 4, and 7 inch maxima being respectively 98° C., 74°, 38° for 95 pounds pressure closed, compared to 99°, 72°, 38° for 10 pounds pressure open. The number of minutes elapsing after steam was turned on before these maxima were reached are almost equally comparable, being 32, 54, and 151 minutes for the 95-pound pressure group and 29, 62, and 155 minutes for the 10-pound pressure group. All three depths reached a maximum of 100° where the soil was steamed 40 minutes at 20 pounds pressure, the lengths of time to attain the maximum being 15, 32, and 38 minutes. The maximum sustained temperatures for these groups are closely correlated with the maximum temperatures.

Wart spores have not been found deeper than 8 inches below the surface of the soil. It was found that steaming for 85 minutes at 90 pounds pressure did not always prevent the occurrence of wart. It is thought that this was because the method of regulating the steam supply was inaccurate, so that the failure was due to a steam flow below that intended. The temperature table shows that at 10 pounds pressure 100° C. was reached in 70 minutes at the 7-inch depth. At the rate of penetration shown, the 8-inch depth should have reached approximately 100° at the end of 85 minutes of steaming. The maximum sustained temperature at the above 7-inch depth was 96°. Hence the maximum sustained temperature at 8 inches following 85 minutes of steaming at 10 pounds pressure should be near the boiling point also. The results obtained in wart control when correlated with recorded temperatures for the treatments used indicate that a sustained temperature somewhere near 100° is necessary to kill the wart sporangia. On this basis only plots treated for 75 minutes at 10 pounds or for 40 minutes at 20 pounds could be expected to show freedom from the disease. In fact, the maximum temperatures obtained at 7 inches in the other groups show that the treatments they represent would be utterly ineffective against organisms with only a moderate tolerance of heat—unless those organisms occurred or remained viable only in the upper 2 or 3 inches of soil. Steam was used, full force (135 pounds), on one pan area in the steam garden, the temperature at 7 inches going up to 99° in 20 minutes. The temperatures fell rapidly, however, so that the "maximum sustained temperature" was an average of temperatures with a minimum of 88°. The average for one-half hour was 93°.

In studying Table V, it should be borne in mind that penetration of heat is relatively rapid as long as it is being supplied in quantity in the steam pan, but that as soon as the steam is shut off the penetration slows up. The shorter the steaming period the longer it takes

for the 7-inch depth to reach its maximum temperature. In several cases the temperature at the 7-inch depth fluctuated, seeming to indicate that for a time the soil received heat from above faster than it was transmitted to the soil below and that later the heat was transmitted much faster than received so that the temperature became lower. The only indication of such a condition in the table is at the 7-inch depth of the first set of temperatures, 95 pounds for 25 minutes. In this case the maximum reached was 29° C. Ten minutes later the temperature was still 29°, but at the end of 20 minutes and of 30 minutes it was 28°, returning to 29° at the end of 45 minutes. So far as could be determined, the fluctuations though slight were not due to errors in the readings, which were made to tenths of a degree. The data for all four of the pan areas used in making these averages show such fluctuations.

The table shows that in most cases the temperature began to fall at the 1-inch depth almost as soon as the steam was shut off. At the 4-inch level the temperature continued to rise for several minutes after steam was shut off, except where the 100° C. was reached before the steam was shut off. At the 7-inch level the temperature continued to rise for a considerable time after steam was shut off, except where a temperature of 100° had previously been reached. The temperatures at all depths usually became somewhat uniform within 4 hours after steam was turned on, regardless of whether steamed 25 or 40 minutes, the temperatures being somewhat higher than the initial temperatures.

A comparison of the temperatures obtained with 10 pounds and with 20 pounds of steam flowing for 40 minutes shows a closer approach to the 1:2 ratio than might have been expected. With 10 pounds of steam the maximum temperatures at 1 inch, 4 inches, and 7 inches averaged 52° C. higher than the initial temperature, while with 20 pounds of steam they averaged 86.5° higher. With 10 pounds of steam the maxima were reached in 29 minutes, 62.5 minutes, and 155 minutes, as compared to 15 minutes, 32 minutes, and 38 minutes at 20 pounds steam pressure. The rates of fall in temperature after the maxima were reached, in minutes per degree, were 3.3 minutes, 6.0 minutes, and 58.0 minutes at 10 pounds as compared to 2.5 minutes, 3.4 minutes, and 3.4 minutes at 20 pounds of steam.

Ten pounds of steam for 75 minutes gives approximately the same "maximum sustained temperature" as does 20 pounds of steam for 40 minutes, viz., 100°, 99°, and 97° C. as compared to 99.5°, 99°, and 96°. It will be noticed, however, that the rates of rise and fall are approximately twice as rapid in soil treated at 20 pounds. Where a high temperature for a relatively short period would be effective the higher pressure would seem desirable on account of the saving in time.

SUMMARY

During the period of steaming, heat rapidly penetrates soil under a steam pan. After steam is turned off, heat penetrates slowly. Hence the shorter the steaming period the longer the time required for the deeper soil to reach maximum temperatures. Steam-pan treatments necessary to exterminate the potato-wart organism heat the topmost 8 inches of soil to approximately 100° C.

Doubling the steam pressure almost doubles the rate of penetration of heat in the soil under the steam pan. The rate of increase

in the temperature of soil under the steam pan varied from 1° C. in 0.17 minute to 1° in 19 minutes; the rate of decrease in temperature for the periods during which data were recorded varied from 1° in 2.5 minutes to 1° in 107 minutes; the maximum temperatures reached varied from 100° to 29°; the maximum temperatures maintained for 30 minutes varied from 100° to 28°. The variations in each case depend on the pressure of the steam used, the length of the treatment, and the depth in the soil.

PART III.—THE PENETRATION OF SOIL FUNGICIDES

INTRODUCTION

Chemical soil disinfection necessarily implies contact between the sterilizing agent and the organism whose destruction is desired throughout its vertical distribution. Some chemicals, such as copper sulphate, are adsorbed or removed from water solutions before penetrating soil more than 2 or 3 inches. Other chemicals, such as bichloride of mercury, react with the soil chemicals and rapidly lose their toxic character. If the approximate penetration ability of chemicals were known, much unnecessary work would be avoided, since it would be possible to eliminate field tests of chemicals that would not penetrate the soil and to use only such strengths and quantities of other chemicals as would be reasonably sure to penetrate to a sufficient depth. Moreover, if a chemical failed to penetrate soil readily but had other merits, it might be tested by working it into the soil.

An examination of the chemical literature available and consultation with chemists failed to disclose the existence of the needed data on the soil penetration of chemicals. Simple tests for use in determining the depth of penetration of possible fungicidal chemicals were next sought.

Many fungicides are complicated compounds. The actual chemical state of the effective element in these compounds is not always known. In the case of these and of some of the simple compounds the tests in common use may be for one of the elements making up the compounds. Positive tests might then be obtained, even though the nature of the compound had been changed by reactions with the soil and its effectiveness lost. In other words, the writers did not know whether or not the tests available could be depended on for use in soil solutions. For many of the compounds with which the writers wished to experiment, no test, or at least no usable test, was available. Any test requiring prolonged individual attention would not be usable. The possession of penetration data offered such possibilities of increased efficiency in the prosecution of the soil sterilization studies that it seemed desirable to investigate the subject as thoroughly as possible, in spite of the difficulties involved.

HISTORICAL

Heretofore soil sterilization work has been largely confined to the control of seed-bed and greenhouse diseases and under specific conditions. Little data on the amount of penetration obtained are given in the reports. Gilbert (4), in speaking of formaldehyde sterilization of tobacco seed beds for *Rhizoctonia* and *Thielavia* root rot, recommends drenching the soil with formaldehyde, using 1 part of formaldehyde to 150 to 200 parts of water, three-fourths to

1 gallon of solution per square foot. "The solution should be put on with a watering pot with a rose and distributed as evenly as possible over the bed so as to thoroughly wet the soil to a depth of a foot." The writers' experiments, carried out with various types of soil in different moisture conditions, show that there is considerable variation in the amount of solution necessary for thorough wetting of the soil to a given depth. Hartley (7, 8, 9), the leading authority on damping-off diseases of forest-tree seedlings, recommends the application of 2 to 7 c. c. of concentrated sulphuric acid in 500 to 1,000 c. c. of water per square foot of seed bed. He found that the amount of acid needed varies with the kind of soil, and the quantity of water required varies with the soil moisture. Hartley and Pierce say (8, p. 7): "The disinfectant must be dissolved in sufficient water to permit its distribution through the soil to a depth of several inches, but within certain limits the concentration of the solution as applied does not appear to be an important factor." Most of the coniferous seed beds had sandy soils. Where the seed-bed soil was heavy and the addition of acid caused marked effervescence, indicating alkalinity, it was found that copper-sulphate solution gave better results than the sulphuric-acid application.

Several others have carried on experimental work on the control of seed-bed and greenhouse diseases by complete or partial soil sterilization.

Halstead (6) tried a variety of chemicals in soil treatments, but the applications of one-tenth gallon per square foot were inadequate for penetration beyond the surface few inches at most. None of the publications consulted contain more than incidental and very general statements as to penetration. There were no data available that would enable one to plan soil treatments intelligently or with even a reasonable degree of assurance that important and variable factors had been taken into account.

The present paper records the progress made in securing fundamental data on the penetration of soil fungicides. The work involved the determination of satisfactory tests for the presence of certain chemicals in treated soil and the penetration of certain chemicals in different soils under different moisture conditions when different strengths and amounts of solution were used. Two improvements were made in the method of application of a water solution by the use of a cheap chemical to protect an expensive one in the solution, and by the application of these chemicals in part of the water followed by the remainder of the water to secure a more even distribution of the toxic chemical.

APPARATUS, METHODS, AND MATERIALS

A few preliminary experiments on formaldehyde penetration were made in the field near Freeland, Pa., in 1920. Most of the experiments were carried out in the laboratory at Washington, D. C. The use of laboratory methods and equipment made it possible to carry on the work during the winter and multiplied the amount and increased the accuracy of the data it was possible to secure.

After consultation with some members of the staff of the Bureau of Soils, equipment was ordered, including soil tubes, sieves and shaker, compactors, balances, chemicals, and ordinary laboratory supplies of glassware.

The soil tubes used were practically all 2 by 15 inch glass tubes. A few brass tubes were secured, but owing to the corrosive action on them of some of the chemicals tested their use was limited. In order to have the soil evenly compacted, standard compactors were secured. The compactor used for the brass tubes has a propeller in the tube and gives the tube quarter-inch drops as it is rotated. The other compactor is of the springboard type recommended for use with glass tubes. With this compactor the tube rests on the middle of the springboard while a weight is dropped from a definite height onto the end of the springboard. After a number of tests it was decided that six drops of the weight from the top of its guide rod gave a uniform compaction with the soils used. Eight-inch sieves of different sizes and an electric sieve shaker were secured. This shaker has a semirotary and undulatory motion, with a decided jar given the sieves when revolved. A series of sieves can be used at the same time.

SOILS USED

The soil used was almost entirely Manor loam from Chevy Chase, D. C., or Leonardtown silt loam from Farlee, Va. A few tests were run with potting soil from the greenhouses, but this was found to be too variable. Manor loam is a common soil type near Washington, D. C., containing 20 per cent or more of clay and having a moisture equivalent of 20.1 per cent.⁹ Leonardtown silt loam, common in nearby Virginia and Maryland, contains 15 per cent or less of clay and has a moisture equivalent of 23.8 per cent. All Manor loam soil used was from a single load of soil, and the Leonardtown silt loam was all taken from an area only a few feet in diameter. These soils were naturally uniform, and each lot was made even more uniform by thorough mixing. The region about Freeland, Pa., is transitional between the Volusia and the DeKalb series of soils. Both types are silt loams with some tendency to acidity. The coal-miners' gardens in which the wart disease occurs are conglomerations of ashes, cinders, coal dust, and soil, so that soil types for the region do not necessarily indicate what may actually be found in a given garden.

PREPARATION OF SOIL TUBES

Soil to be tested in tubes was run through a screen (quarter-inch mesh) to remove any roots, stones, and large lumps. The soil for each tube was weighed. The amount of soil used in a tube depended somewhat on the character of the soil and the tests to be run. In some cases 500 grams of the loose soil filled a tube, or the amount of penetration to be expected made the use of more soil unnecessary. When the soil was in such condition that a larger amount of the loose soil would go into a tube and the amount of penetration expected made it advisable, 600 grams per tube was used. In either event the soil was compacted as described above. The height of the soil columns secured was reasonably uniform. The heights of soil columns as given were taken after treatment. The number of tubes used in a set varied greatly. The penetration secured was so uniform for similar tubes that only three or six of a set of similar tubes were ordinarily tested in any one day. This made it possible to run tests

⁹ Moisture equivalents furnished by John W. McLane of the Biophysical Laboratory, Bureau of Plant Industry

of a number of different treatments at the same time for comparative purposes. To avoid confusion the tubes used in each experiment were numbered consecutively after the experiment was set up.

HOW SOLUTIONS WERE APPLIED

Preliminary tests showed such uniform penetration following applications by simply pouring the proper amounts of solution from a graduate onto the soil in the tubes that no other method of application was tried. In many cases the chemicals to be applied were dissolved in part of the water and then used, the remainder of the water being applied later. For convenience notations were used to indicate this, as follows:

- $1+0=1$ gallon of solution per square foot.
- $1+1=\frac{1}{2}$ gallon of solution per square foot $+\frac{1}{2}$ gallon of water.
- $1+3=\frac{1}{4}$ gallon of solution per square foot $+\frac{3}{4}$ gallon of water.
- $1+7=\frac{1}{8}$ gallon of solution per square foot $+\frac{7}{8}$ gallon of water.
- $(1+1)+0=\frac{1}{2}$ gallon of solution per square foot $+\frac{1}{2}$ gallon of solution.

In other words, these figures are based on applications totaling 1 gallon per square foot, the first figure showing the number of parts of the water used in making up the chemical solution applied and the second figure showing the number of parts of water applied later. These figures are used particularly for applications of mercuric chloride, and, unless otherwise specified, indicate the application of 1 gallon of one-half of 1 per cent mercuric chloride plus $2\frac{1}{2}$ per cent sodium chloride per square foot, or its equivalent.

DETERMINATION OF SOIL MOISTURE

Although only approximate soil-moisture determinations were required, they were made with considerable care. The crucibles used for soil-moisture determinations were of known weight. Weights to balance the crucible to be used were first placed on one side of the analytical balances and a 50-gram weight added. The crucible was then removed from the desiccator and put on the other scale pan with soil added to balance. After 48 hours in the electric oven at 95° C. the crucible was placed in a desiccator to cool and was then reweighed. The weight in milligrams was recorded. The per cent of moisture equals weight lost divided by the dry weight of soil. While the time the samples were kept in the oven often varied somewhat, the percentages of moisture obtained are accurate enough for our purposes.

METHOD OF HANDLING SOIL SAMPLES TESTED FOR THE PRESENCE OF CHEMICALS

After tubes of soil treated with chemical solutions had remained in position for the desired length of time the soil was removed an inch at a time and each inch tested for the presence of the chemical applied. Before removing any soil from a tube a wax pencil was used to mark the tube off into 1-inch sections, starting from the top. The bottom inch or fraction of an inch of soil was then carefully removed with a spatula and stirred up with 50 c. c. of water in a 250 c. c. beaker. The number of the tube from which this soil section was taken and the number of inches from the top of the soil column to this particular section was marked on the beaker. The same procedure was followed until each inch of soil in the tube had been similarly treated. When the soil in the beaker had settled, the clearer liquid on the surface was decanted and filtered into test tubes. This

filtrate was then tested for the presence of the chemical with which the soil had been treated. After some experience it was often possible to estimate the depth of penetration, so that only a few tests for each tube were necessary.

THE CHEMICAL TESTS USED

TEST FOR FORMALDEHYDE

The first test made was one for formaldehyde. A number of tests, particularly Schiff's, were tried without success. Schryver's test as given by Haas and Hill (5 p. 59) was found to give good results in general. The method is as follows:

To 10 c. c. of the liquid to be tested add 2 c. c. of a 1 per cent solution of phenylhydrazine hydrochloride freshly made up and filtered; then add 1 c. c. of a 5 per cent solution of sodium ferricyanide, also freshly made up, and 5 c. c. of hydrochloric acid; a brilliant magenta color is produced. The test is a very delicate one and will detect quantities of formaldehyde varying from 1 part in 1,000,000 to 1 part in 100,000. Acetic aldehyde gives no color with this reagent.

In preliminary work¹⁰ with soil solutions to which known quantities of formaldehyde had been added, successful tests were obtained in dilutions as great as 1 part in 1,250,000. Yet in some cases positive tests were not obtained in dilutions greater than 1 part in 250,000. Slight differences in the weighing or measuring of chemicals, in their uniformity, or in the age of the solutions, may have been responsible for the variations. The color produced is brilliant only when there is sufficient formaldehyde present. When nearing the limit of dilution for positive tests the color produced is very faint pink. In the case of a few soil samples a deep green color was produced, completely screening the red color if such were produced. This green color was not encountered in soil except where foreign material was present. It seemed to be due to the presence of iron in certain forms.

TEST FOR MERCURY AND COPPER

Hydrogen sulphide was used as a test for mercury bichloride and copper sulphate. For convenience water was charged with hydrogen sulphide gas and some of this added to the clear filtrate obtained from the soil solution. When the amount of metal present was small the precipitate would cause only a faint browning of the solution. It had been feared that this test would be unusable owing to possible action of soil chemicals. There was no visible evidence that the hydrogen-sulphide tests were affected by soil chemicals at any time.

Manufacturers are now testing a number of mercury compounds as possible disinfectants and fungicides. Some of these compounds do not form Hg-ions in water solutions and therefore are not precipitated by H₂S. One of the manufacturing companies suggested that we ignite the soil, boil gently in acetic acid, filter, and precipitate with ammonium sulphide. The presence of iron in the soils caused an excessively heavy precipitate which obscured any mercury precipitate that may have been present. It had been suggested that these non-ionizing compounds should penetrate better than mercury bichloride. Without a usable test for their presence in soil solutions the writers have been unable to test this theory.

¹⁰ L. O. Overholts of Pennsylvania State College, performed many of the preliminary experiments with the formaldehyde tests.

TEST FOR KEROSENE

No chemical test could be found for kerosene, nevertheless some experiments were run with it. The penetration of the kerosene was tested by three methods. The soil sample on removal from the tube was smelled, then part of it was placed on a sheet of white paper and the remainder put in water. Kerosene makes a transparent spot on paper and makes a film of oil on water; or, if present in larger amounts, forms drops of oil on the surface of the water. Repeated tests checked by several persons indicated that the odor test was shorter and perhaps more accurate than the others. The odor test was checked by the oil-film-on-water test each time. These tests may be far from accurate. In many cases the odor of the soil was changed markedly for two inches below the point which gave a characteristic kerosene odor. Whether this change in odor indicated the presence of small amounts of kerosene or of some penetrating constituent of the kerosene such as vapor or soil-water film, the writers do not know.

TEST FOR ALCOHOL

To test for the presence of alcohol a few crystals of iodine were dissolved in the filtered soil solution and a few drops of a dilute solution of sodium hydroxide were added. The test tube was then gently heated if necessary. If alcohol was present an odor of iodoform was given off, and if the amount of alcohol was not too small, yellow crystals of iodoform were precipitated.

TEST FOR QUA-SUL

The chemical test used to determine the presence of Qua-sul was a simple precipitation test. When concentrated hydrochloric acid was allowed to drop into dilute solutions of Qua-sul, free sulphur was precipitated.

TEST FOR SODIUM CHLORIDE

To test for the presence of salt in soil solutions a dilute solution of silver nitrate was used. The addition of a few drops of the silver nitrate solution to the soil solution filtrate caused the formation of a white precipitate if sodium chloride was present.

PRELIMINARY PENETRATION TESTS

PENETRATION OF FORMALDEHYDE

Almost immediately after the chemical test for the presence of formaldehyde in soil solutions was found, experiments were begun to determine the penetration of formaldehyde in soil in the experimental gardens at Freeland, Pa. Some plots 1 yard square were laid out and treated with formaldehyde at the rate of $\frac{1}{2}$ gallon per square foot. Samples from near the center of these small plots were tested. All tests of soil taken from a depth greater than 4 inches were negative for most plots. Occasionally formaldehyde was found below 4 inches, in one or two cases as deep as 8 to 9 inches. A few preliminary experiments were carried out in the laboratory at Washington, D. C., with tap water to determine the approximate amounts of soil required to fill the tubes used and of water and time required

to secure percolation of the water through the tubes. Using this preliminary data as a basis, laboratory tests with formaldehyde were planned.

The first tests were made with six brass tubes in series. The tubes were filled and then compacted with the compactor for metal soil tubes. The column of soil was usually $14\frac{1}{2}$ inches deep after compaction. Just enough solution was supplied to keep it at the overflow level in all tubes. The time was recorded when solution was turned on the tubes and again when percolate began to drip from the bottom of each tube. The percolate was tested for formaldehyde and gave a strong positive test in each case. Three strengths of formaldehyde were used, viz, $\frac{1}{20}$ per cent, $\frac{1}{4}$ per cent, and 1 per cent. The rate of percolation in air-dry (3 to 4 per cent moisture) Manor loam soil varied from 7.8 to 11.4 minutes per inch, averaging 9.6 minutes per inch for the 23 tubes used. The rate of percolation in potting soil was much more rapid. Six tubes of potting soil with 3.13 per cent of moisture showed percolation at the rate of 4 minutes per inch. Six tubes with 11.3 per cent moisture showed percolation at the rate of 3 minutes per inch.

PENETRATION OF COPPER SULPHATE

In tests with 1 per cent copper sulphate solution in potting soil (6 tubes) containing 7.6 per cent of moisture the average rate of percolation was 4 minutes per inch. With a 2 per cent solution of copper sulphate, using Manor loam (6 tubes) containing 6.3 per cent of moisture, the rate of percolation averaged 11 minutes per inch. No positive test for copper was secured in the percolate from any of these tubes.

PENETRATION OF ALCOHOL

Alcohol, 47 per cent, in one test with six tubes of potting soil containing 4.45 per cent moisture, showed percolation at the rate of 6.5 to 9.5, averaging 8 minutes per inch. The percolate gave positive tests for alcohol in each case.

PENETRATION OF QUA-SUL

Qua-sul, a proprietary sulphur compound, was tried at 1 per cent strength on six tubes of potting soil with 8.7 per cent moisture. Percolation was at the rate of 3.8 to 8.8, averaging $5.3+$, minutes per inch. No sulphur could be precipitated in the percolate. The potting soil used was taken from the mixing bench.

If variations in the make-up of the soil in different tubes was responsible for the wide variations in rate of percolation, these variations in composition did not show in a casual examination of the soil.

AMOUNT OF SOLUTION REQUIRED FOR PERCOLATION

The amount of solution required to percolate through the tubes of soil was determined from the increase in weight. The tubes were weighed dry and again after the percolate started to drip, but without allowing the soil to drain. The increased weight in grams was assumed to equal the number of cubic centimeters of water held.

The maximum amount of solution held by any one of 30 tubes of potting soil was 300 c. c., the minimum 227 c. c., the average 258 c. c. The maximum amount of solution held by any one of 17 tubes

of Manor loam soil was 333 c. c., the minimum 294 c. c., the average 323 c. c. These figures show far less variation than the rates of percolation. Most sets of six tubes varied little within the set—in one case only 6 c. c. Most sets were of air-dry soil. As the tubes are 2 inches in diameter, applications of 258 c. c. per tube would be equal to approximately 3 gallons per square foot to secure percolation through 13½ inches of potting soil and of 323 c. c. per tube would be approximately 4 gallons per square foot to secure percolation through 14½ inches of Manor loam soil. At the time these laboratory tests were started the field applications had not exceeded 2 quarts of formaldehyde solution per square foot, and tests of field-treated soil had indicated that such applications could not be depended on to penetrate more than 4 or 5 inches. A few tubes were set up with soils to which definite amounts of water had been added, and formaldehyde solution was then added to each tube at the rate of 2 quarts per square foot. The results were exceedingly erratic. To other sets of tubes applications were made at the rate of 1 gallon per square foot. Of 9 tubes set up with Manor loam put through an 8-mesh-to-the-inch sieve, 2 tubes showed penetration 9 to 10 inches, 5 tubes 8 to 9 inches, and 2 tubes 7 to 8 inches at the end of 1 day. These results indicated that it would be necessary to use applications of 1 gallon or more per square foot in order to secure the 8 inches of penetration required to reach all wart spores in the soil.

PENETRATION OF FORMALDEHYDE COMPARED WITH THAT OF MERCURIC CHLORIDE

The experiments with formaldehyde indicated that the penetration of formaldehyde in water solutions practically coincided with the penetration of the water of the solution. The test for formaldehyde while sensitive and accurate and relatively simple is far more tedious than the simple precipitation test with H_2S for mercury. Preliminary determinations were made to see if the H_2S precipitate test could be successfully used in soil solutions. No positive results were obtained with this test at any depth in either potting soil or Manor loam treated with 1 gallon per square foot of 1 to 1,000 mercuric chloride solution. Three tubes of potting soil were then given applications of 1 per cent mercuric chloride at the rate of 3 gallons per square foot. A faint mercury test was obtained in the percolate from each tube. These tests indicated rapid removal of the mercury from the solution as it passed through the soil. To retard this removal of the mercury, sodium chloride was added to the mercuric chloride solution at the rate of 5 parts of salt to 1 part of mercuric chloride. Salt was first added to mercury in our field treatments because Hartley (?) reported that the combination was particularly severe on green plants when used in soil treatments. It was thought possible it would be equally severe in its action on the wart fungus. In studying the penetration of mercuric chloride it seemed wise to test the penetration of the solution actually used in the field experiments. The penetration was found to be very much better when salt was added to the solution. Manor loam with a moisture of 9.5 per cent and potting soil with a moisture of 6.8 per cent were used for parallel sets of tubes treated with 1 per cent formaldehyde and with 2 per cent mercuric chloride plus 10 per cent sodium chloride, at the rate of 200 c. c. per tube (nearly

2½ gallons per square foot). Six glass tubes were used for each of the four sets and the soil columns were from 9 to 10½ inches deep. Strong positive tests for formaldehyde or for bichloride of mercury were obtained in the percolate of all tubes. Percolation of the formaldehyde solution was more rapid than that of the mercuric chloride solution. In Manor loam the formalin solution percolated in 29 to 35 minutes, averaging 32 minutes, while mercuric chloride percolated in 38 to 54 minutes, averaging 49 minutes. In potting soil the formalin solution percolated in 4 to 27 minutes, averaging 8 minutes, while mercuric chloride percolated in 12 to 14 minutes, averaging 13 minutes.

Manor loam soil containing 8.2 per cent of moisture, and potting soil containing 6.8 per cent of moisture were used in two parallel sets of tubes treated with 2 per cent mercuric chloride and with 2 per cent mercuric chloride plus 10 per cent sodium chloride at the rate of 2 gallons per square foot. Three hours after the tubes were treated the percolate from the 12 potting-soil tubes gave positive tests for mercury. In three days the Manor loam tubes were tested and positive tests secured in the bottom inch in each case. There was no percolate from the Manor loam tubes. The height of the soil columns varied from 9¼ to 10½ inches at the time of testing.

PENETRATION OF MERCURIC CHLORIDE

STANDARD APPLICATION OF MERCURIC CHLORIDE

The foregoing experiments showed that mercuric chloride with its simple and rapid H_2S test might be successfully used in penetration studies. The strength of solution was modified on the basis of further experiments, some of which are recorded below. One gallon of ½ per cent $HgCl_2$ + 2½ per cent $NaCl$ solution, or its equivalent, per square foot was adopted as the standard application. In a comparative test of applications of 1 gallon of ½ per cent mercuric chloride + 2½ per cent sodium chloride versus ¼ per cent mercuric chloride + 1¼ per cent sodium chloride, tubes tested at the end of two days and others at the end of three days showed 9 to 10 inches penetration for the stronger solution, and 8 to 9 inches penetration for the weaker solution. The soil in these tubes contained 15.1 per cent moisture. That mercuric chloride alone in solution does not give satisfactory penetration at similar strengths and applications is indicated by a test on soil containing 11.9 per cent of moisture. One gallon of 1 per cent mercuric chloride solution showed 6 to 7 inches penetration in one day and the same in two days, and ½ per cent solution showed 4 + inches penetration in one day and 5 to 6 inches in two days.

The above tests were on Manor loam. Later Leonardtown silt loam containing 6.23 per cent moisture was treated with 2 quarts of 1 per cent $HgCl_2$ + 2 quarts of water. At the end of 2 days 5 tubes showed 7 inches penetration, in 8 days 4 + inches, and in 11 days 5 to 6 inches. This would seem to indicate the gradual removal of the bichloride of mercury after penetration.

Reducing the proportion of salt from five times to twice the amount of mercuric chloride reduced penetration (Table VI). Applying the solution in two parts did not give as good results as applying the chemicals in half the water and applying the other half subsequently.

TABLE VI.—*Effect of reducing proportion of NaCl to HgCl₂ in 1+1 and (1+1)+0 applications*

Method of application	Solutions applied		Penetration	
	First application	Second application	In 3 days	In 4 days
1+1	1 per cent HgCl ₂ +5 per cent NaCl, 2 quarts per square foot.	Water, 2 quarts per square foot....	<i>Inches</i> 8+	<i>Inches</i> 9
(1+1)+0.....	½ per cent HgCl ₂ +2½ per cent NaCl 2 quarts per square foot.	½ per cent HgCl ₂ +2½ per cent NaCl, 2 quarts per square foot.	7 to 8	7 to 8
1+1.....	1 per cent HgCl ₂ +2 per cent NaCl, 2 quarts per square foot.	Water, 2 quarts per square foot....	7 to 8	8 to 9
(1+1)+0.....	½ per cent HgCl ₂ +1 per cent NaCl, 2 quarts per square foot.	½ per cent HgCl ₂ +NaCl, 2 quarts per square foot.	7 to 8	6 to 7

* See page 332, "How solutions were applied" for further explanation of these symbols. Each figure is the average for 3 tubes. Soil used, Manor loam containing 10.5 per cent of moisture.

STANDARD TREATMENT

On the basis of these and other preliminary experiments 1 gallon per square foot was adopted as the standard application and the amount of chemicals required to make 1 gallon of ½ per cent HgCl₂+2½ per cent NaCl solution was adopted as the standard quantity of chemical to be applied per square foot. It was not deemed wise to spend the time necessary to fix the amount of chemicals more accurately, especially in view of the fact that it seemed certain to vary with different soils.

PENETRATION OF HgCl₂ IN POTTING SOIL

Penetration of mercuric chloride in potting soil was so erratic that experiments with potting soil were discontinued. Using 1 gallon of solution (or of solution plus water) the penetrations, as determined by tests with H₂S, were as follows: In air dry (2 per cent moisture) potting soil penetration varied from none at the end of five days to 7 to 8 inches at the end of one day (18 tubes); in soil containing 11.4 to 14.8 per cent moisture the maximum penetration secured was 2 to 3 inches (36 tubes); in soil containing 17.6 to 17.8 per cent moisture penetration varied from 7 to 8 inches to 10 to 11 inches (36 tubes). In 13 of the tubes filled with soil containing 11.4 or 14.8 per cent moisture no positive test was obtained at any depth. Two of these were tested at the end of one day. Evidently the mercuric chloride was removed from solution or else its character changed very soon after application. Whether the differences in results were due to varying amounts or types of fertilizers in the soil used or to other causes was not determined.

PENETRATION OF HgCl₂+NaCl SOLUTIONS IN MANOR LOAM AS SHOWN BY H₂S TESTS

As shown in Tables VII and VIII, the penetration of mercuric chloride in solutions ¹¹ containing sodium chloride and applied at the rate of 1 gallon per square foot was determined in soil containing different percentages of moisture. In many of the experiments the

¹¹ Unless otherwise stated standard treatments were given.

chemicals were applied in part of the water and the remainder of the water was applied subsequently. In soil containing 4 per cent or less of moisture the average penetration was about 7 inches; in soil containing 10 to 16 per cent or 20 to 23 per cent of moisture the penetration averaged 8 inches; while in soil containing 17 to 19 per cent the penetration averaged somewhat more than 8 inches. The depth of penetration seems to have been about the same whether the chemicals were applied in all or in part of the water.

TABLE VII.—*Penetration of mercury in mercuric chloride plus sodium chloride solutions in Manor loam, as shown by hydrogen sulphide. Average penetration (inches) at different percentages of soil moisture of applications totaling 1 gallon per square foot*

In soil containing 4 per cent moisture									
Proportion of solu- tion + water	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Number of tubes
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	
1+0 -----	7 to 8		7-		7-				9
1+1 -----	7	7+	7+	8-	8-				48
1+3 -----		6+	7+	7+	7+			8 to 9	27
1+7 -----			7		7+				6

In soil containing 10 to 16 per cent moisture									
1+0 -----		8	8+	7 to 8			8 to 9		18
1+1 -----		8-	8-	8+			8 to 9		30
1+3 -----		7+	8-	6 to 7		7 to 8			18
1+7 -----		7+	8-	8 to 9		8+			12

In soil containing 17 to 19 per cent moisture									
1+0 -----	8-	9	9 to 10	9 to 10			9 to 10		24
1+1 -----	8 to 9	8+	8+	9	9 to 10	9	9 to 10	9 to 10	40
1+3 -----	9-	9	8+	8+	8+	9-	8+	9 to 10	70
1+7 -----	8-	9-		8+	8 to 9	8 to 9	8+		36

In soil containing 20 to 23 per cent moisture									
1+0 -----		6+	7 to 8	7-	8-				12
1+1 -----		7	7 to 8	7 to 8		7 to 8		8 to 9	14
1+3 -----		8-	9 to 10	8+		9			12
1+7 -----		7-	8-	8 to 9		8 to 9			12

SUMMARY ^b

Per cent moisture	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Number of tubes
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	
4 -----	7-	7-	7+	8-	7+			8 to 9	90
10 to 16 -----		8-	8-	8-	9-	8-	8 to 9		78
17 to 19 -----	8-	9-	8+	8+	8+	8+	9-	9 to 10	170
20 to 23 -----		7-	8-	8-	8-	8-		8 to 9	50
Total -----									388

^a 1+0=1 gallon ½ per cent HgCl₂+2½ per cent NaCl per square foot.
1+1=½ gallon 1 per cent HgCl₂+5 per cent NaCl+½ gallon water per square foot.
1+3=1 quart 2 per cent HgCl₂+10 per cent NaCl+3 quarts water per square foot.
1+7=1 pint 4 per cent HgCl₂+20 per cent NaCl+7 pints water per square foot.
See p. 332, "How solutions were applied."
^b This combines the data contained under 4, 10 to 16, 17 to 19, and 20 to 23 per cent moisture.

TABLE VIII.—Number of tubes giving positive tests for mercury with the hydrogen sulphide test, at the various depths on each day after application to Manor loam of 1 gallon per square foot of mercuric chloride plus sodium chloride solutions, or of 1 gallon of solution plus water ^a

In soil containing 4.3 per cent or less moisture

Depth of penetration	1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	Total number of tubes
<i>Inches:</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	<i>Tubes</i>	
6 to 7	3	2	3	—	1	—	—	—	9
7 to 8	6	9	23	3	13	—	—	—	54
8 to 9	—	1	7	6	9	—	—	3	26
9 to 10	—	—	—	—	1	—	—	—	1

In soil containing 10 to 16 per cent moisture

6 to 7	3	—	—	3	—	—	—	—	6
7 to 8	3	21	15	6	—	3	—	—	48
8 to 9	—	13	19	15	8	2	6	—	63
9 to 10	—	5	15	3	4	1	—	—	28

In soil containing 17 to 19 per cent moisture

7 to 8	6	1	3	4	—	2	—	—	16
8 to 9	13	9	6	13	8	11	15	—	75
9 to 10	5	9	11	18	7	10	15	2	77
10 to 11	—	2	1	1	—	1	—	—	5

In soil containing 20 to 23 per cent moisture

6 to 7	—	5	—	—	—	—	—	—	5
7 to 8	—	4	7	5	—	4	—	—	20
8 to 9	—	2	2	5	—	6	—	3	18
9 to 10	—	1	3	1	—	2	—	1	8
Total	—	—	—	—	—	—	—	—	459

^a This table includes the tubes from some sets used in special experiments which were not included in Table VI. The amounts of chemicals used per square foot in these special experiments varied from the equivalent of those required for one gallon of $\frac{1}{2}$ per cent $\text{HgCl}_2 + 2\frac{1}{2}$ per cent NaCl , the standard application. Since no increased penetration seemed to result from such increased amounts of chemicals it seemed permissible to include them here.

The data are arranged to show the number of days elapsing from the time of application of the solution until the penetration tests were run. It will be noted that the maximum penetration was usually not obtained in less than three days. As previously stated penetration was determined by testing soil an inch at a time. A positive test in the soil sample from the 8 to 9 inch depth was considered 8 inches in tabulating, even though the actual penetration may have been nearer 9 inches. Hence one may find an average penetration of 8 inches shown in the tables, whereas the penetration for the tubes included averaged more than 8 inches. In two tubes in which the penetration was $8\frac{1}{4}$ and $8\frac{1}{2}$ inches, positive tests would be secured in the 8 to 9 inch soil section and both tubes would be listed as showing 8 inches of penetration. A tube in which penetration was barely 8 inches would give a positive test in the 7 to 8 inch soil section and hence would be listed as showing 7 inches of penetration. The average of these three tubes, $8 + 8 + 7$, would appear as 8 — inches in the table instead of $8\frac{1}{4}$ inches. Although this may seem inexact it must be borne in mind that for absolute extermina-

tion of a soil organism the minimum penetration is the important figure and also that owing to the time and labor involved tests were necessarily made only by inches.

In Table VIII the data are arranged to show the number of tubes giving positive tests at various depths each day. The daily increase in the proportion of positive tests at lower depths is very marked in the dry soil. The proportion of positive tests at 6 to 7 inches and at 7 to 8 inches is 1:2 at the end of the first day, becomes 1:4 in two days, 1:8 in three days, and 1:13 at the end of five days. At the same time there is a marked increase in the number of positive tests at 8 to 9 inches. The proportions do not change so widely and regularly at the other soil moistures, but the change is quite marked.

PENETRATION OF MERCURY IN $\text{HgCl}_2 + \text{NaCl}$ SOLUTIONS IN LEONARDTOWN SILT LOAM

Penetration of $\text{HgCl}_2 + \text{NaCl}$ solutions in Leonardtown silt loam was studied in the same way as penetration in Manor loam and similar tabulations of the data obtained are given in Tables IX and X. As in the Manor loam, the penetration in the drier soil averaged about 7 inches, this depth only being exceeded by part of the tubes where the application was made 1+1, that is, the chemicals were applied in half the water and an equal amount of water was applied later. The penetration in soil containing 8 to 15 per cent of moisture averaged 8 inches or a little more, only slightly less than the average penetration in soil containing 15 to 21 per cent of moisture. Penetration in soil containing 43 per cent of moisture was extremely rapid as well as approximately 3 inches deeper than in soil containing 21 per cent or less of moisture. Of the 34 tubes, 21 gave positive tests for mercury in the solution that percolated through the tubes, and 7 gave positive tests in the bottom inch of soil. The figures given therefore do not represent the absolute maximum of penetration for such wet soil. As in Manor loam, the maximum penetration was usually not obtained in less than three days, and depth of penetration did not seem to be particularly affected by applying the chemicals in part of the water and then applying the remainder of the water.

In Table X the penetration data are arranged to show the number of tubes giving positive tests at each depth on successive days after the solutions were applied. The successive increases in the proportion of positive tests at lower depths are not so marked as in the Manor loam, although it is evident, particularly in the soil containing 8 to 15 per cent of moisture.

DISCUSSION OF PENETRATION OF $\text{HgCl}_2 + \text{NaCl}$ SOLUTIONS

EFFECT OF SOIL TYPE AND SOIL MOISTURE

A comparison of the data in Tables VII and IX shows slightly better penetration in air-dry Manor loam than in air-dry Leonardtown silt loam. In Manor loam containing 10 to 16 per cent moisture the penetration was practically the same as in Leonardtown silt loam containing 8 to 15 per cent moisture. In Manor loam penetration was better in soil containing 17 to 19 per cent moisture than in soil containing 20 to 23 per cent moisture. In Leonardtown silt loam

TABLE IX.—Penetration of mercury in mercuric chloride +sodium chloride solutions in Leonardtown silt loam. Average penetration (inches) at different percentages of soil moisture of applications totaling 1 gallon per square foot

In soil containing 1.3 to 4.3 per cent moisture									
Proportions of solution and water	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Number of tubes
	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	
1+0-----		6-			6+				6
1+1-----		7-	7+	8	7-		8 to 9		33
1+3-----			7-		6 to 7				6
1+7-----			5 to 6		6+				6
In soil containing 8 to 15 per cent moisture									
1+0-----	7-	7+	8				8-		12
1+1-----	8	8-	7 to 8	8+	8+	8 to 9	8 to 9		42
1+3-----	8 to 9	8-		8+	8 to 9	8 to 9	8 to 9		24
1+7-----	8-	8-		8+	8-	8+	8 to 9		24
In soil containing 15 to 21 per cent moisture									
1+0-----		8 to 9	8 to 9	8+		8 to 9			12
1+1-----		9-	9	9	9-	9-			75
1+3-----		9-	8 to 9	8 to 9		8+		8-	12
1+7-----		8 to 9	8 to 9	8-		8+			12
In soil containing 43 per cent moisture									
1+1-----	10+	11 to 12		11-	10-				11
1+3-----	11-	11 to 12		12-	12-				12
1+7-----	12 to 13	11+		12-	12 to 13				11
SUMMARY ^b									
Moisture in soil (per cent)	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Number of tubes
1 to 4-----		6+	7-	8-	6+		8		51
8 to 15-----	7+	8-	7+	8+	8+	8+	8-		102
15 to 21-----		8+	8+	8+	9-	8+	8-	8-	111
43-----	11	11+		11+	11+				34
Total-----									298

^a See "How solutions were applied," p. 332, and footnote of Table VII.
^b This combines the data contained under 1.3 to 4.3, 8 to 15, 15 to 21, and 43 per cent moisture.

there did not seem to be any real change in the depth of penetration as the percentage of moisture increased from 15 to 21 per cent. In the case of the Manor loam the soil containing 20 to 23 per cent had reached or exceeded the moisture equivalent percentage (20.1 per cent) for that soil, whereas in the Leonardtown silt loam the moisture equivalent is 23.8 per cent, or somewhat higher than the maximum moisture in the 15 to 21 per cent group of tubes. It has been suggested that there may be some correlation between the moisture equivalent and the penetration obtained. This has not been investigated. The only tests of Leonardtown silt loam containing more than the moisture equivalent were the tubes containing 43 per cent of moisture. These tubes were set in 8 inches of water for two days and then drained a short time (part of one day) before being treated.

TABLE X.—Number of tubes giving positive tests for mercury at the various depths on each day after application to Leonardtown silt loam of 1 gallon per square foot of ½ per cent mercuric chloride + 2½ per cent sodium chloride solution, or its equivalent in chemical solutions plus water

In soil containing 4 per cent or less of moisture									
Depth of penetration	1 day	2 days	3 days	4 days	5 days	6 days	7 days	12 days	Total
Inches	Tubes	Tubes	Tubes	Tubes	Tubes	Tubes	Tubes	Tubes	Tubes
4 to 5		1							1
5 to 6			3		1				4
6 to 7		3	3		9				15
7 to 8		6	8	3	5				22
8 to 9			3	3			3		9

In soil containing 8 to 15 per cent moisture									
6 to 7	1	3							4
7 to 8	6	7	3	1	1	2	1		21
8 to 9	8	16		18	10	5	11		66
9 to 10		1		5	1	1			8
10 to 11						1			1

In soil containing 15 to 21 per cent moisture									
7 to 8				1		1	1	2	5
8 to 9		12	15	16	4	9	2	7	65
9 to 10		9	6	10	8	8			41

In soil containing 43 per cent moisture									
9 to 10					1				1
10 to 11	3			2					5
11 to 12	3	7		3	3				16
12 to 13	3	1		4	4				12
Total									298

As shown in Tables IX and X, exceptionally good penetration was obtained in these tubes. In general the depth of penetration increases as the percentage of moisture increases, though not regularly. Differences in penetration are not always shown by the penetration figures as taken. A set of tubes in which actual penetration was 7¼ inches is listed as 7 to 8 inches and in these summaries is counted as 7 inches, just as another set in which actual penetration may be 7⅞ inches. The actual depth of penetration in two sets of tubes may be the same, and at the same time strong tests may be obtained for one set of tubes and very weak tests for the other set. Difference in the strength of tests was noted, particularly in studying the penetration where chemicals were applied in part of the water and the remainder of the water added later.

PENETRATION WHERE ALL CHEMICALS WERE APPLIED IN PART OF THE WATER

Tables VII and IX indicate that penetration into the soil was about equally good whether all of the water was used in making up the chemical solution or whether only part was used and the rest applied later.¹² On the whole the figures indicate slightly better

¹² The effect of the amount of time elapsing between application of the chemical solution and application of the water is discussed later.

penetration where part of the water was applied later. The chemical tests emphasized this difference. Where all of the water was applied in the chemical solution, the test for mercury was often very faint in samples from near the point of maximum penetration. The strength of the test was apt to be variable, not only between different tubes but within a tube. When part of the water was withheld and applied after the application of the chemical solution, stronger and more uniform tests were obtained at points nearing the maximum depth of penetration, and the tests from different parts of the same tube were more nearly uniform.

What proportion of the water could be applied as such is not known. In our experiments three proportions were tried: 1 + 1, or equal parts of solution and water; 1 + 3, or one part solution to three parts of water; and 1 + 7, or one part solution to seven parts of water. As shown by Tables VII and IX, no one of these proportions is superior to the others in every case. In practically every case all three were equal to or superior to the 1 + 0 applications in the depth of penetration obtained. In applying solutions and water to the tubes the liquid was simply poured from the graduate. In some cases, especially where the amount of solution was small, it is quite possible most of the solution was applied to one side of the tube and that the water was poured on the other side of the tube. This would tend to reduce the amount of chemical forced down the tube by the water. So far as the writers are aware, the limiting factors in reducing the amount of water used in making up the solution would be the amount necessary to put the chemicals into solution readily and the amount of solution necessary to insure even distribution over the soil surface.

PENETRATION OF STRONG SOLUTIONS OF $\text{HgCl}_2 + \text{NaCl}$ WHEN IMMEDIATELY FOLLOWED BY AN APPLICATION OF WATER AND WHEN FOLLOWED LATER BY AN APPLICATION OF WATER

As previously shown, the depth of penetration of mercury in $\text{HgCl}_2 + \text{NaCl}$ solutions is about the same whether all the water is used in making up the solution or part of it is withheld and applied immediately after the application of the chemical solution, but the latter method gives a more even distribution of the mercury in the soil. This latter method offers another advantage in that no special apparatus is required in applying water while special apparatus and special care are required and therefore additional expenses incurred, in using mercuric chloride, on account of its corrosive action. Sometimes rain might supply at least a part of the water. Whether the water is applied with a hose or falls as rain it is important to know whether this water application must follow the chemical solution immediately or whether the water may be applied at some later and perhaps more convenient time. That final penetration of the mercury is not adversely affected by delaying the application of the water is indicated by the results given in Table XI, summarizing the penetration data from four sets of tubes. In every case the maximum penetration was the same for treatments differing only in the amount of time elapsing between the application of the first and second parts of the treatment. While these experiments gave favorable penetration with delayed applications, it is conceivable that a soil might react so rapidly with the bichloride of mercury that the delay would result in a serious reduction in the strength of the solution.

TABLE XI.—Effect of delaying second part of applications made in two parts, 1+1, 1+3, 1+7, (1+1)+0

Method of application	Time elapsing between two parts of application	Depth of penetration in inches							Soil moisture
		Time after application of first part until test was made							
		21 hours	2 days	3 days	4 days	5 days	6 days	7 days	
		<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Per cent</i>
1+1 ^a -----	10 to 15 minutes-----	7 to 8	8 to 9	^b 8+	8 to 9	-----	-----	-----	14 to 16
1+1-----	17 hours-----	6 to 7	7 to 8	8-	8 to 9	-----	-----	-----	14 to 16
1+0-----	Only one application-----	-----	7 to 8	7+	7 to 8	-----	-----	8 to 9	10
1+1-----	17 hours-----	-----	7+	7+	8 to 9	-----	-----	8 to 9	10
1+1-----	10 to 15 minutes-----	-----	8-	-----	-----	9-	-----	-----	10
1+1-----	18 hours-----	-----	8+	-----	-----	9-	-----	-----	10
(1+1)+0-----	10 to 15 minutes-----	-----	8-	-----	-----	8 to 9	-----	-----	10
(1+1)+0-----	18 hours-----	-----	7 to 8	-----	-----	8 to 9	-----	-----	10
1+3-----	10 to 15 minutes-----	-----	-----	-----	9-	8+	9-	8+	18.6
1+3-----	20 hours-----	-----	-----	-----	8-	8 to 9	9-	8 to 9	18.6
1+7-----	10 to 15 minutes-----	-----	-----	-----	8 to 9	8 to 9	8+	8+	18.6
1+7-----	20 hours-----	-----	-----	-----	8 to 9	8 to 9	8+	8 to 9	18.6

^a For explanation of symbols, see "How solutions were applied," p. 332, and footnote of Table VII.
^b Three tubes of each lot were tested each day. Where all three tubes gave positive tests (H₂S precipitation method) to the same depth, that depth is indicated by 7 to 8, 8 to 9, etc.; when the depth varied, plus or minus signs are used, e. g., 8+=2 tubes gave positive tests at 8 to 9 inches and the third at 9 to 10 inches; 8-=2 tubes gave positive tests at 8 to 9 inches, and the third at 7 to 8 inches. Soil, Manor loam.

RESULTS OBTAINED FROM EXPERIMENTS COVERING SPECIAL PHASES OF SOIL PENETRATION BY CHEMICALS, PARTICULARLY BY MERCURY IN HgCl₂+Na Cl SOLUTIONS

In addition to the foregoing experiments, the data from which are included in Tables VI to XI, a number of experiments were conducted to secure data on other phases of the problem of chemical penetration in soil. These include experiments to determine: (1) The effect of the size of soil particles on the depth of penetration; (2) the effect of a high water table on the depth of penetration; (3) the amount of lateral penetration of mercury in the soil; (4) the upward penetration of mercury in soil; (5) the depth of penetration in tubes which had been allowed to stand for three months after being set up and before being treated; (6) the effect of passage through soil on the toxicity of a solution of HgCl₂+NaCl; and (7) the relation of depth of penetration to the amount of liquid applied.

EFFECT OF SIZE OF SOIL PARTICLES ON THE PENETRATION AND PERCOLATION IN THEM OF CHEMICAL SOLUTIONS

It is well known that different soils are composed of different proportions of various sizes of soil particles. In gravelly soils, composed of very coarse particles, water penetrates with great ease and rapidity. In clay soils, composed of very fine particles, water penetrates with difficulty and very slowly. Samples of Manor loam and of Leonardtown silt loam were sieved and tests made to determine penetration in the various sizes of soil particles obtained. The soil samples were air dried, rolled fine and sieved with an electric sieve shaker. The sieves used had 20, 40, 60, 80, 100, 150, and 200 meshes per inch. It was found that a considerable proportion of the soil went through all of these sieves, that practically all soil passing through the 60-mesh sieve passed also through the 80-mesh sieve so that very little soil of this texture was available for the experiments, and that the proportion of soil held by the other sieves varied considerably.

The chemical solutions used in testing penetration in the sifted soil were $\text{HgCl}_2 + \text{NaCl}$ (1 + 1) and 1 per cent formaldehyde (commercial solution). The penetration data secured in these sieved soils is tabulated in Table XII. As shown in this table penetration was better in the coarse than in the fine soil. However it does not seem likely, from the results secured, that variations in the proportions of the various sizes of particles would be sufficient to cause any important variations in the depth of penetration in soils of the same general type.

TABLE XII.—Effect of size of soil particles on penetration

Soil	Manor loam, air dry		Leonardtown silt loam, 4.9 per cent moisture
	Mercuric chloride + sodium chloride 1+1 ^a penetration in 3 days	1 per cent formaldehyde 1+0 ^a penetration in 17 to 18½ hours	Mercuric chloride + sodium chloride 1+1 ^a penetration in 3 days
	Inches	Inches	Inches
Whole.....	6 to 7 ^b (2) 7 to 8 ^b (1).....	7 to 8 (3).
20 to 40 ^c	8 to 9 (9).....	8 to 9 (6).....	7 to 8 (3).
40 to 60.....	6 to 7 (6) 7 to 8 (3).....	7 to 8 (3) 8 to 9 (3).....	7 to 8 (3).
60 to 80 ^d	6 (1) to bottom of column.
80 to 100.....	6 to 7 ^d (2).....	6 to 7 (6).....	6 to 7 (1) to bottom of column.
100 to 150.....	6 to 7 (1).....	6 to 7 (6).....
100 to pan ^e	4 to 5 (3).
150 to 200.....	6 to 7 (4).....	5 to 6 (2), 6 to 7 (3).....
200 to pan.....	5 to 6 (4).....	5 to 6 (1), 6 to 7 (5).....
Leonardtown silt loam whole.	6 to 7 (1), 7 to 8 (2).....

^a 1+1=½ gallon 1 per cent HgCl_2 +5 per cent NaCl +½ gallon water per square foot. 1+0=1 gallon 1 per cent formaldehyde (commercial) per square foot.
^b Numbers inclosed in parentheses indicate the number of tubes showing the depth of penetration indicated.
^c The first figure gives the number of meshes per inch in the sieve the soil passed through and the second figure is the number of meshes per inch in the sieve holding the soil.
^d The sifted soil was not equally divided among the various sizes. Very little was held in the 80-mesh sieve particularly, and not enough in some other sieves to run a full series of tests.
^e The 150 and 200 mesh sieves were not available when the Leonardtown silt loam was sifted.

That the rate of penetration of a chemical solution in soil particles of different sizes may vary considerably is indicated by an experiment with formaldehyde in Manor loam. The six brass tubes were set up and 700 grams of sieved soil compacted in each tube. A 0.05 per cent solution of commercial formaldehyde was supplied to these tubes until percolate dripped from the outlet of all tubes. The percolate gave a strong positive test for formaldehyde in every case. The mesh of the sieves is given as in Table XII, together with the height of the soil column through which the solution passed and the time elapsing after the solution was turned on until percolate appeared. The weight of solution held at the end of the test by each tube of soil is also given. The results of the experiment are shown in Table XIII. The figures in the table show that the volume of the soil did not vary greatly and bore no relation to the rate of penetration. The penetration in the smallest particles was so slow that inspections were reduced to one per hour and a small amount of percolate had accumulated in the beaker under the drip when the inspection was made at the end of the tenth hour. The weight of the solution held by the 100 to 150 tube was exactly twice that held by the 20 to 40 tube. This ratio did not hold for the rate of penetration given above nor for the depth of penetration of one gallon of solution as given in Table XII.

TABLE XIII.—Percolation of 0.05 per cent formaldehyde solution in soil particles of different sizes

Soil	Height of soil column	Time to percolate	Water held by soil ^a
	<i>Inches</i>		<i>Grams</i>
20 to 40 ^b	13	16 minutes.....	264
40 to 60.....	14½	do.....	436
80 to 100.....	15¾	32 minutes.....	506
100 to 150.....	15¾	2 hours, 1 minute.....	528
150 to 200.....	14¾	2 hours, 55 minutes.....	407
200 to pan.....	14¾	9 to 10 hours.....	453

^a The last column shows the gain in weight during treatment.
^b Sieves as in Table XII. Soil=Manor loam, air dry, 700 grams per tube. A strong positive test for formaldehyde was obtained in the percolate from each tube.

EFFECT OF A HIGH-WATER TABLE ON THE PENETRATION OF MERCURY IN THE SOIL

The influence of a high-water table on the penetration of mercury in HgCl₂ + NaCl solutions was tested by setting tubes of soil in beakers and adding water to the beakers. The results of these tests as shown in Table XIV were very erratic. The first set of tubes was set up and the application made to the tops of the tubes about 20 minutes after the water had been added to the beakers, and the visible water line showed that the water had risen 5 inches in the tubes. The penetration was about the same as in the tubes treated in the usual manner. The second set of tubes was set up and the chemical solution and water applied to the soil tubes almost immediately after the water had been added to the beakers. In this case the penetration was not nearly so great as in tubes treated in the usual way. The beakers were removed from half of the high-water table tubes at the end of one day to permit drainage. Penetration in these tubes then improved so that a week after the treatments were given they nearly equaled the tubes treated in the ordinary manner. It was thought that the poor penetration of the high-water table tubes in this set was due to the fact that the chemical solution only penetrated far enough to meet the upcoming flow of water. If the water is added to the beakers beforehand the upward flow will become nearly stationary in a few minutes in damp soil, the flow being slower in dry soil. With the glass tubes used the rise of the water line was easily observed.

A third set of tubes was prepared and water added to the beakers and allowed to stand overnight. The soil, being dry, had taken up practically all of the water so that more was added. In this case the tubes treated regularly did not show as good penetration as those with the high-water table. Removal of the beakers did not seem to improve penetration in this set.

The results of the fourth experiment confirmed those already obtained. The tubes receiving applications some time after water was added to the beakers showed good penetration, and tubes receiving applications at the same time water was added to the beakers showed poor penetration. An additional lot of tubes receiving applications before water was added to the beakers showed poor penetration. The results of these experiments indicate that a high water table, if stable or receding, would not prevent adequate penetration. A rising water table would be expected to interfere seriously with penetration.

TABLE XIV.—Effect of high water table ^a on the penetration of mercury in mercuric chloride plus sodium chloride solution

Method of application	High-water table or otherwise	Depth of penetration on succeeding days after treatment						Soil moisture
		1 day	2 days	3 days	4 days	6 days	7 days	
Set 1		Inches	Inches	Inches	Inches	Inches	Inches	Per cent
1+3 ^b	High-water table.....			^c 8½	9			17.9
	Checks, regular applications.....			^c 9 to 10	9 to 10			17.9
Set 2								
1+3.....	High-water table.....			5 to 6	5 to 6		5 to 6	12.3
	Checks, regular applications.....			7+	7+		8+	17.3
	High-water table removed.....			5+	6 to 7		8—	12.3
Set 3								
1+3.....	High-water table.....		7 to 8	9—	8 to 9	9—		10.2
	Checks, regular applications.....		7 to 8	7+	6 to 7	7 to 8		10.6
	High-water table removed.....		8—	8+	9—	8+		10.2
Set 4								
1+1.....	High-water table before applications.	8 to 9	8 to 9	8—	^d 7+			10.66
	High-water table after applications.	6+	6 to 7	5+	6 to 7			10.66
	High-water table with applications.	6—	5 to 6	6 to 7	6 to 7			10.66

To simulate a high-water table, tubes of soil were set in beakers and water added to the beakers. Set 1: 100 c. c. water was added to beakers and soil was treated 20 minutes later, after water line showed 5 inches up from bottom. Set 2: 100 c. c. water was added to beakers and soil was treated 15 minutes later. The beakers were removed from part of tubes about 24 hours after treatment. Set 3: 100 c. c. water was added to beakers; 17½ hours later 50 c. c. more water was added to beakers and soil was treated. Beakers were removed from under part of tubes next day. Set 4: One lot had 83¼ c. c. water added to beakers and was tested 2 hours and 40 minutes later. Second lot, soil was treated and water added to beakers 2 hours and 30 minutes later. Third lot, soil received chemical solution and beakers received water at same time and soil received its water application 2 hours later.

^b For explanation of symbols see p. 332 and footnote of Table VII.

^c Each penetration figure given is the average for 3 tubes.

^d Two tubes showed 8 to 9 inches penetration and the other 6 to 7 inches.

LATERAL PENETRATION IN SOIL OF MERCURY IN MERCURIC CHLORIDE PLUS SODIUM CHLORIDE SOLUTIONS

Sterilization of soil by application of chemical solutions can not be accomplished in soil full of stones or other impenetrable obstacles unless the chemical reaches all spores in the soil under such obstacles. A few tests of such lateral penetration were made with 6-inch flower pots, 3⅜-inch petri-dish bases for obstacles, Leonardtown silt loam containing 12 per cent of moisture, and the regular 1+1 application of HgCl₂+NaCl solution. Thirty-six pots were partly filled with soil, inverted petri-dish bases placed on this soil in a horizontal position and the pots filled. In 12 pots the petri-dishes were at a depth of 2 inches; in another set of 12 the petri dishes were at a depth of 3 inches; and in the third set at 5 inches. The pots were treated with 1 per cent HgCl₂+5 per cent NaCl at the rate of one-half gallon per square foot and followed by an equal amount of water. Four days later the soil was carefully removed from above the petri dishes, then the petri dishes were removed and the soil that had been protected by the petri dish was tested for the presence of mercury. No positive tests were obtained. Owing to the sloping sides of the pots a relatively small circle of soil was left uncovered by the petri dish even at 2 and 3 inches, while at 5 inches the petri dish almost rested on the sides of the pot.

TABLE XV.—*Lateral penetration, under an obstacle in the soil, of mercuric chloride plus sodium chloride solution as shown by hydrogen sulphide and silver nitrate tests*

	Depth to obstacle	Lateral penetration under obstacles at 1-inch and 6-inch depths on successive days		
		After 2 days	After 3 days	After 4 days
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
H ₂ S test for HgCl ₂	1	1 to 2 (4) °-----	0 to 1 (2), 1 to 2 (2).	0 to 1 (3), 1 to 2 (1).
	6	0 to 1 (1), none (3)	None (4)-----	None (4).
AgNO ₃ test for NaCl.....	1	1 to 2 (4)-----	1 to 2 (4)-----	1 to 2 (4).
	6	1 to 2 (4)-----	1 to 2 (4)-----	1 to 2 (4).

° Numbers in parentheses show the number of pots, with the lateral penetration indicated.
Pots, 8-inch; petri dishes, 3½-inch; soil, Leonardtown silt loam containing 13.5 per cent moisture.

A second set of 24 pots was prepared in the same manner as the above but with the use of 8-inch instead of 6-inch pots and by placing the petri-dish bases at 1-inch and at 6-inch depths. As shown in Table XV, there was some penetration of mercury under all of the petri dishes at the 1-inch depth, while only one positive test for mercury was obtained under the petri dishes at the 6-inch depth. The diminishing number of positive tests under the center of the petri dishes at 1 inch would seem to indicate the gradual change of the mercury to compounds not precipitated by the H₂O + H₂S test used. Positive tests with AgNO₃ throughout the soil under the petri dishes at both the 1-inch and the 6-inch depths indicated a thorough penetration by the NaCl.

Just what is responsible for the apparently poor penetration of the mercury is not known. The percentage of HgCl₂ was made as low as seemed safe to insure penetration in soil free from obstacles. It is well known that lateral penetration in soil is poor. It seems quite likely that most of the mercury reaching the top of the petri-dish base remains there. Even if it all worked over the edge of the petri dish, much of it would probably go down rather than laterally, under the obstacle. It would seem probable that the amount of mercury moving back under the petri dish would be so small that reactions with soil chemicals would soon change its form so that it would not react to the H₂O + H₂S test used. Much the same thing may occur in tubes of dry soil treated and allowed to stand until there is no longer a sharp line between the moist treated soil and the dry untreated soil but a very gradual transition from one to the other. Soil from the transition zone is likely to give only negative results when tested for mercury.

UPWARD PENETRATION OF MERCURY

A single experiment was run to determine the approximate upward penetration of mercury as compared to downward penetration. Of 24 tubes filled with Leonardtown silt loam containing 4.26 per cent moisture, 12 tubes were treated in the usual way, 1 + 1, and the other 12 were treated by standing them in beakers and pouring the solution and the water into the beakers. The water was not added until the solution had been taken up by the soil. Three tubes from each lot were tested on the second, third, fourth, and seventh days. The

regular tubes gave positive tests at 7 to 8 inches from the top on the second day and at 8 to 9 inches on the remaining days. All of the upward penetration tubes gave positive tests at 7 to 8 inches from the bottom except two tubes which gave positive tests at 6 to 7 inches on the fourth day.

PENETRATION OF MERCURY IN SOIL ALLOWED TO STAND THREE MONTHS

If soil could be sterilized without being freshly cultivated it would often save expense and facilitate treatment. When only newly cultivated soil can be treated, every rain delays the work until the soil is again in condition to permit cultivation. It is much harder to haul loads of chemical solutions over newly cultivated soil. A single experiment was set up to give an indication of the penetration to be expected in soil permitted to stand without cultivation. As it seemed quite possible there would be a difference between soil receiving most of its moisture as rain and a soil receiving its moisture through subirrigation, the tubes were divided into two sets.

Forty-eight tubes were filled with Manor loam and set in beakers; 100 c. c. water was added to the tops of half of the tubes; 18 hours later 50 c. c. water was poured into the beaker under each tube; the remaining 24 tubes had 100 c. c. water added to the beaker for each tube and another 100 c. c. water added to the beaker three days later. Three months later these tubes were treated, half of each lot being treated 1+1 and the other half 1+0. Three tubes from each lot were tested on the second, third, fifth, and sixth days. As shown in Table XVI, the penetration of 1+0 was much better than 1+1 in the tubes that had received 100 c. c. of water on top and 50 c. c. of water at the bottom, while in tubes receiving 100 c. c. twice at the bottom the 1+1 penetrated much better than the 1+0. No explanation of this variation has been suggested. Penetration in the poorer lot in each case was about equal to that obtained in freshly prepared air-dry soil.

TABLE XVI.—*Penetration of mercury in mercuric chloride and sodium chlorid solutions in tubes of Manor loam allowed to stand three months after having water added to them*

Water added 3 months before treatment	Chemical treatment given	Penetration on successive days after treatment			
		2 days	3 days	5 days	6 days
		<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
100 c. c. water applied to top and 50 c. c. to beaker at bottom of each tube.....	1+1	7 to 8 (3)	7 to 8 (3)	7 to 8 (3)	7 to 8 (2)
	1+0	6 to 7 (1) 7 to 8 (2)	7 to 8 (1) 8 to 9 (2)	8 to 9 (1) 9 to 10 (2)	8 to 9 (1) 9 to 10 (2)
100 c. c. water applied to beaker +100 c. c. to beaker at bottom of each tube.....	1+1	8 to 9 (3)	7 to 8 (1) 8 to 9 (2)	8 to 9 (2) 9 to 10 (1)	8 to 9 (2) 9 to 10 (1)
	1+0	6 to 7 (2) 7 to 8 (1)	6 to 7 (3)	6 to 7 (2) 7 to 8 (1)	7 to 8 (3)

* For explanation of symbols see p. 332 and footnote of Table VII, p. 339. 100 c. c. of water is equal to 1½ gallons per square foot of soil surface in the tubes used.

• Numbers inclosed in parentheses show the number of tubes giving positive tests at the depths indicated.

TOXICITY OF MERCURIC CHLORIDE PLUS SODIUM CHLORIDE SOLUTIONS AFTER PASSING THROUGH SOIL

The effect of passage through soil on the toxicity of the $HgCl_2 + NaCl$ solution was tested with only a few tubes of heavily infected soil from Freeland, Pa. Sixteen tubes were prepared in the usual manner on January 26, 1922. Thirteen tubes were treated 1+1 and 3 tubes were treated with water plus water to serve as checks. In eight days 3 of the 13 treated tubes were tested for mercury penetration. Positive tests were secured in each inch of soil in all three tubes from the surface to a depth of 9 inches. Twenty-six days after treatment the soil was removed from all tubes and used to inoculate tubers. Pots were partly filled with uninfected soil, a tuber laid on the soil, and 1 inch of soil removed from one of the tubes was scattered over the tuber and soil. The pot was then filled to the proper height with uninfected soil. At the end of three months the potato plants were examined for wart.

As shown by Table XVII, the 90 pots inoculated with soil, from the surface to a depth of 9 inches, in the treated tubes remained free from wart infection. Of 29 pots inoculated with soil from treated tubes but from below the 9-inch depth to which the mercury had been shown to penetrate, 6 pots, or 21 per cent, showed wart. Of the 35 pots inoculated with soil from the check tubes 10 pots, or 29 per cent, showed wart. A second series of tubes gave almost identical percentages of infection. Apparently the treatment of soil with 1 per cent $HgCl_2 + 5$ per cent $NaCl$ solution at the rate of $\frac{1}{2}$ gallon per square foot and followed by an equal amount of water is effective to the maximum depths of penetration in the Freeland soil.

TABLE XVII.—*Effect of soil penetration on toxicity to the potato-wart organism of mercuric chloride plus sodium chloride solution as shown by infections of potato plants grown in pots inoculated with soil from different depths in treated tubes*

Depth from which in-oculation soil ^a was taken	Inoculated with water-treated soil		Inoculated with 1+1 ^b treated soil		Depth from which in-oculation soil ^a was taken	Inoculated with water-treated soil		Inoculated with 1+1 ^b treated soil	
	Plants ex- amined	Plants show- ing wart	Plants ex- amined	Plants show- ing wart		Plants ex- amined	Plants show- ing wart	Plants ex- amined	Plants show- ing wart
Inches	Num- ber	Num- ber	Num- ber	Num- ber	Inches	Num- ber	Num- ber	Num- ber	Num- ber
0 to 1	3	1	10	0	6 to 7	3	1	10	0
1 to 2	3	1	10	0	7 to 8	3	0	10	0
2 to 3	3	1	10	0	8 to 9	3	2	10	0
3 to 4	3	0	10	0	9 to 10	3	1	10	1
4 to 5	3	1	10	0	10 to 11	3	1	10	4
5 to 6	3	0	10	0	11 to 12	2	1	9	1

^a Wart-infected soil from Freeland, Pa., containing 12.8 per cent moisture.
^b For explanation of symbol, see p. 332 and footnote of Table VII, page 339.

RELATION OF SOIL PENETRATION OF WATER SOLUTIONS TO THE AMOUNT APPLIED

With the glass soil tubes used the penetration of water could be readily determined at any time in most soils, particularly if dry, by

the difference in color between the soil dampened by the water applied and that not yet so dampened. The penetration of mercury and of formaldehyde in water solutions was found to agree largely with this visible penetration of the water carrying them. Hence the approximate penetration of given amounts of these solutions could be determined by noting their visible penetration in soil tubes or by determining the visible penetration of like amounts of water. Wart spores have not been shown to be present below the 8-inch level in soil, and this depth is usually reached by applications of 1 gallon per square foot, the amount adopted as a standard application in our experiments. It seems quite possible that deeper penetration would sometimes be desirable and in very dry soil the amount of solution applied might need to be increased to insure a uniform penetration of 8 inches. If the depth of penetration should be found to be in direct proportion to the amount of the application, the required amount of increase in application any time the penetration was found to be inadequate could be computed immediately instead of awaiting the data from experimental tests. Since the penetration of mercury and formaldehyde solutions seems to approximate the penetration of water, water alone was used in these tests.

In order to determine whether penetration was directly proportional to the amount of liquid added or whether it became greater or less (in proportion to the quantity applied) as the quantity applied was increased, 36 tubes of Leonardtown silt loam containing 5.73 per cent moisture were used. Applications of water at the rate of 1, 2, 3, 4, 5, and 6 quarts per square foot were made, using 6 tubes for each lot. At the end of one day the penetration in each tube was determined without disturbing the tubes by measuring the distance from the surface to the very distinct water line. In tubes treated with 6 quarts per square foot the water was found to have penetrated to the bottom of the tubes. An additional quart per square foot was added to each of the other tubes immediately after the measuring was completed. One day later the penetration in these tubes was again measured. The average increase in penetration, as shown by the last column in Table XVIII, is relatively uniform. The range of increases, however, in the next to the last column shows a wide variation, from $1\frac{1}{4}$ to $3\frac{3}{4}$ inches per tube. It was thought possible that the tubes showing the greatest penetration this second day might be those that showed penetration below the average on the first day, or that the conditions that caused more than average penetration on the first day would cause an increased penetration on the second day in the same tube. Inspection of the figures failed to bear out either hypothesis. A curve plotted from any one of the columns of averages would be as regular as could be expected with such a small number of tests. The maxima in the first two range columns also show almost regular curves. In practical work the minimum penetration is the important consideration. The data of this table indicate that the minimum varies greatly and the average penetration is sometimes nearly an inch greater than the minimum. Due allowance for this would have to be made in planning treatments to secure penetration to a definite depth.

The penetrations secured in this experiment are much higher than were secured in regular treatments of Leonardtown silt loam of low moisture content (Table X).

TABLE XVIII.—Penetration of various amounts of water in Leonardtown silt loam containing 5.73 per cent moisture

Amount added per square foot	Penetration					
	After 1 day		One quart per square foot added after measuring and another measurement made the following day		Increased second day over first	
	Range °	Average	Range	Average	Range	Average
Quarts	Inches	Inches	Inches	Inches	Inches	Inches
1	2¾ to 3½	3½	5 to 6½	5½—	1¾ to 2¾	2½
2	4¾ to 5½	5¼	7 to 8¼	7¾—	2¼ to 2¾	2½
3	7½ to 8	7½+	9¼ to 10½	10+	2¼ to 3	2½
4	9½ to 10½	10—	11¾ to 12½	12½	1¼ to 3	2¼
5	9½ to 12	11	12½ to 14¼	13½—	1½ to 3¾	2¾
6	13¼ to 14	13½	Penetration through column without addition.			-----

° Each of the six lots consisted of six tubes.

SOIL PENETRATION OF OTHER CHEMICALS TESTED

SOIL PENETRATION OF WATER SOLUTIONS OF COPPER SULPHATE

Copper sulphate is such an old and much-used fungicide that a few penetration tests were run with it notwithstanding the well-known fact that copper sulphate solutions soon lose their copper sulphate when applied to soil. A 1 per cent solution of CuSO₄ failed to give a positive test after percolation¹³ through 14 inches of potting soil. A 2 per cent solution failed to give a positive test after percolation through 14½ inches of Manor loam. Six tubes were used in each of the above sets. Other tests with varying strengths of CuSO₄ seemed to indicate that attempts to get penetration with CuSO₄ alone in a solution were certain to fail. Since the addition of NaCl to solutions of HgCl₂ insures mercury penetration, it was thought that similar results might be secured by adding a salt to CuSO₄ solutions.

The first test of CuSO₄ with a salt was made with six tubes of air-dry Leonardtown silt loam, three treated 1+1 with 2 per cent CuSO₄+10 per cent NaCl and three treated 1+1 with 2 per cent CuSO₄+5 per cent Na₂SO₄. At the end of four days the tubes treated with CuSO₄+NaCl gave positive tests for copper at 6 to 7 (2 tubes) and 7 to 8 (1 tube) inches and the tubes treated with CuSO₄ gave positive tests at 4 to 5 (1 tube) and 5 to 6 (2 tubes) inches. The results seemed very encouraging, especially those obtained with NaCl, so that the experiments were continued.

The results obtained with Na₂SO₄ were so poor that few tests were made with it.

The penetration of CuSO₄+NaCl was tested in 74 additional tubes with Leonardtown silt loam containing from 10 to 21 per cent moisture. Applications were made at the rate of 1 gallon per square foot in each case. The penetration obtained in these tubes ranged from 3 to 4 inches up to 9 to 10 inches, distributed as follows: 3 to 4 inches (4 tubes), 4 to 5 (12 tubes), 5 to 6 (19 tubes), 6 to 7 (27 tubes), 7 to 8 (11 tubes), 9 to 10 (1 tube). The penetration secured did not warrant the hope that the copper sulphate and salt solution could be expected to give adequate soil penetration to permit its use as a fungicide for potato wart.

¹³ In percolation tests tubes were kept continuously supplied with solution until liquid began to drop at the bottom of the tubes.

PENETRATION OF QUA-SUL

Qua-sul is a proprietary alkaline sulphur compound said to have given good results in various types of treatments. Enough 1 per cent solution of Qua-sul was supplied to six tubes of potting soil to secure about 50 c. c. of percolate from each tube. All tests for sulphur in this percolate were negative. The soil columns were 13 to 14 inches high and about 3 gallons of solution per square foot was used to secure the percolate desired.

PENETRATION OF ALCOHOL

Ordinary grain alcohol was diluted to 47 per cent alcohol and supplied for six tubes of potting soil until about 50 c. c. of percolate was obtained from each tube. The soil columns were 13 to 14 inches high and about 3 gallons of solution per square foot was used to secure the percolate desired. A strong positive test was secured in the percolate from each tube.

PENETRATION OF KEROSENE

The penetration of kerosene was tested, using Leonardtown silt loam with varying amounts of moisture from air-dry to 21 per cent moisture. The data from these experiments have been assembled in Table XIX. It will be seen that in air-dry soil the penetration of kerosene applied at the rate of 1, 2, or 4 quarts per square foot is little better than the penetration of an equal amount of water. In soil containing 10 per cent of moisture the penetration nearly doubled. Further marked increases in penetration are shown at 13, 15, 16.4 per cent of moisture. In soil containing 19 to 21 per cent of moisture the penetration of kerosene applied at the rate of 1 pint per square foot is equal to the penetration of 1 gallon of kerosene or of water in soil containing 1.83 per cent of moisture, and is equal to the penetration of 1 gallon of a water solution in soil containing 19 per cent of moisture. The penetration of 1 quart or more of kerosene in soil containing 19 per cent or more of moisture was not determined as it exceeded the height of the soil columns.

TABLE XIX.—*Penetration, in inches, of kerosene in Leonardtown silt loam on successive days and with different soil moistures*

Soil moisture	Application per square foot	1 day after treatment	2 days after treatment	3 days after treatment	4 days after treatment	5 days after treatment	6 days after treatment	7 days after treatment	8 days after treatment	11 days after treatment
<i>Per cent</i>	<i>Quantity</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
1.8	1 quart.....	^a 2½	2½	3¼	3	-----	-----	-----	-----	-----
1.8	2 quarts.....	4½	5½	5½	5¼	-----	-----	-----	-----	-----
1.8	4 quarts.....	8½	9	9	8¾	-----	-----	-----	-----	-----
10	1 pint.....	-----	2½	2½	-----	-----	-----	-----	-----	-----
10	1 quart.....	-----	4.9	4.9	-----	-----	-----	-----	-----	-----
12	1 pint.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
12	1 quart.....	-----	6¼	6½	6½	6½	6—	5—	7 to 8	4+
13	1 quart.....	-----	-----	6+	7+	7+	8 to 9	9—	10 to 11	8+
15	1 pint.....	-----	-----	10—	10+	11—	-----	8+	-----	-----
16.4	1 quart.....	-----	-----	8¾	9¼	-----	-----	11—	-----	-----
19	1 pint.....	7½	8¼	-----	-----	-----	-----	-----	-----	-----
19	1 quart.....	11 to 12	12+	11 to 12+	11 to 12+	-----	-----	-----	-----	-----
20.8	1 quart.....	-----	10 to 11	10 to 11	10 to 11	10 to 11	-----	-----	-----	-----
(b)	1 quart.....	10+	13 to 14	13 to 14	12 to 13	-----	-----	-----	-----	-----
(b)	2 quarts.....	13 to 14	13 to 14	13 to 14	13 to 14	-----	-----	-----	-----	-----
(b)	4 quarts.....	13 to 14	13 to 14	13 to 14	13 to 14	-----	-----	-----	-----	-----

^a Each figure is the average of 3 tubes.

^b These tubes were saturated with water and then drained before kerosene was added.

Attention is called to the remarkable increase in the penetration of kerosene as the percentage of moisture increased. Kerosene, when not present in excess, has a tendency to spread over the surface of water in a rather uniform film. In very dry soil the film of moisture on the soil particles will be so thin that the exposed surface of the film will extend almost to the points of contact with adjoining particles. A slight increase in the thickness of the water film would cause a very marked decrease of exposed surface in that part of the film in the acute angles near the points of contact. It is conceivable that the increase of 1,000 per cent (1.83 to 19.0 per cent) in the amount of moisture in the soil reduced the amount of exposed water surface so greatly that the 1 pint of kerosene covered this exposed surface with a film of approximately the same thickness as that obtained with 1 gallon in the dry soil. At any rate, it seems likely that changes in the amount of water surface and of surface tension are involved in the changes in kerosene penetration.

FIELD NOTES ON PENETRATION

A considerable number of field plots were treated with various chemicals, some of them liquids or in solution. No systematic effort was made to obtain data on the penetration of these chemicals and solutions. Where penetration was extremely slow or practically failed the fact was usually recorded. These scattered notes are here summarized (Table XX).

TABLE XX.—*Readiness of penetration of chemicals in field plots*

Chemical	Penetration	Chemical	Penetration
Alcohol.....	Good.	Javelle water.....	Poor.
Bichloride of mercury solution.....	Good.	Kerosene.....	Excellent.
Bordeaux.....	Very poor.	Lime-sulphur.....	Fair.
Woburn Bordeaux.....	Poor.	Crude oil.....	Excellent.
Carbolic acid (crude).....	Poor.	Potassium permanganate.....	Poor.
Carbolic acid solution of crystals.....	Poor.	Pyroligneous acid.....	Fair.
Chloride of lime.....	Poor.	Qua-sul.....	Good.
Cleaning solution.....	Very poor.	Sodium chromate.....	Good.
Creosote.....	Good.	Weed killer.....	Very poor.
Formaldehyde.....	Good.		

APPLICATION OF PENETRATION DATA TO FIELD WORK

The study of the penetration of chemicals in the soil was undertaken to enable us to intelligently plan experiments looking to the extermination of the potato wart organism in those regions of Pennsylvania, Maryland, and West Virginia in which it is known to occur. There is a considerable variation in the soils of these regions and in the amount of moisture present in them at different times and seasons. It seemed likely that these and other variable factors would largely influence the possible effectiveness of treatments. The results herein recorded give a valuable insight into the influence of some of these variables and indicate that with data from more extended experiments it would be possible to plan soil treatments with as much assurance as spraying or other remedial or preventive measures are now planned.

The influence of soil type, as shown in Tables VI, VII, VIII, IX, and X, is apparently not very marked in soils of similar nature, although the influence of the percentage of moisture present varied

in the Manor loam and Leonardtown silt loam. The results with potting soil were very erratic, possibly because of the presence of excess humus or of chemical fertilizers. The data on the influence of the size of soil particles indicate that there would have to be a very decided difference in the proportions of very coarse or o very fine particles to markedly influence penetration. Hence if penetration in preliminary tests varies greatly from that expected it seems more likely that the chemical composition of the soil is responsible than that it is due to a variation in physical make-up too slight to be noticeable to the eye.

Soil moisture is constantly changing in the regions where wart is prevalent, because of alternating spells of rain and of dry weather. As the percentage of moisture present has a marked influence on penetration, it would be necessary to determine the soil moisture at frequent intervals in order to give the most economical treatment and to insure proper penetration. Where possible, it would be advantageous to treat the soil while wet or just previous to rainy weather, as less water and materials would be required for adequate penetration. As shown in Tables VII and IX and in the discussion on the effect of applying all of the chemicals in part of the water and following with the remainder of the water as such, the penetration is improved by following this procedure. This indicates that a heavy rainfall within a reasonable time after the application of the strong chemical solution might eliminate the necessity of applying the water. Autumn applications, particularly, might eliminate the expense of much of the water. The application of chemical solutions requires special apparatus and the exercise of considerable care to secure even distribution. Relatively simple apparatus can be used in applying water. Laborers would demand more pay for handling any of the dangerous chemicals than for handling water. One man with a hose could probably apply a much larger quantity of water than one or more men operating an expensive chemical outfit even if there were no delays to refill the chemical outfit.

Much stronger tests are obtained in the soil near the line of maximum penetration when part of the water is applied after the chemicals have been applied. Tests made throughout the soil penetrated indicate a much more even distribution of the chemicals by this method. Hence it might be possible to use a more dilute initial solution at a great saving in chemicals.

The tests of penetration in tubes having a high-water table were made because some infected soil is bottom land with a water table near the surface. It was thought that adequate penetration in such land would be impossible as long as the water table remained high. The results obtained in the laboratory indicate that where this water table was stationary or receding, satisfactory penetration could be secured. The solution might not be effective much below the actual water line, however, as it would soon become greatly diluted even if the depth of penetration were adequate.

Some of the wart-infected soils contain rocks, stones, or débris. If a solution applied to these soils would not penetrate to spores underneath such obstacles, extermination by chemical treatments would become correspondingly complicated. The few experiments on lateral and upward penetration were performed to get information on the amount of penetration under obstacles. The results were

unsatisfactory. In newly prepared dry soil a considerable amount of penetration under an obstacle can be expected. The amount of chemical carried by the solution drawn under the obstacle may easily be insufficient to treat adequately so much soil. In field soil the upward flow of water is retarded by an obstacle with a consequent accumulation of moisture under it. It is doubtful if there would be an adequate flow of solution into this already moist soil. Thorough cultivation would tend to separate the obstacles from the moist soil underneath. Screening and treating soil would be expensive and should be avoided if possible. Further study of the effect of obstacles in soil to be treated should be made. It seems likely that treatments could be made effective without much trouble except where obstacles were larger than 2 inches in diameter or were very numerous. Enough soil contains large obstacles in quantity to make this a problem worthy of further study. Kerosene has such ability to penetrate that it might be useful for ground of this nature.

In the soil-treatment experiments in the field, as in the penetration experiments in the laboratory, the soil was newly cultivated or mixed so that the capillary tubes were broken up and the moisture content made uniform for all parts of the soil likely to be reached by the solution. In a few cases in the field formaldehyde seemed to make especially good penetration in soil that had been undisturbed for some time. The single set of 48 tubes used to test penetration in soil allowed to stand gave peculiar but encouraging results. Further experiments would be necessary to determine the effect of standing under different conditions. If soil could be satisfactorily treated without cultivation an important item of expense could be eliminated. If cultivation were unnecessary, soil could be treated more easily, especially in the spring before dry enough to work or when wet during the summer, as it would be difficult to haul an outfit over soft, wet ground.

Experiments to determine the effect of penetration on the toxicity of the solution to the potato wart organism were very encouraging. For the mercuric chloride plus sodium chloride solution the results indicated that any strength giving a positive test at the required depth, as shown by the hydrogen sulphide test, would be effective. No tests have been run with other fungicides. Of course, other fungi might require a greater strength of solution.

SUMMARY

Study of the penetration of chemicals in the soil is handicapped by lack of tests suitable for determining the presence of chemicals in soil or soil solutions.

Schryver's test was found satisfactory for formaldehyde, hydrogen sulphide for mercuric chloride and copper sulphate, and the characteristic odor for kerosene in soil solutions.

The penetration of formaldehyde is apparently equal to that of the water carrying it in solution even in dilutions of 0.05 per cent of commercial solution.

The penetration of mercuric chloride alone in solution is poor, but it has been found that the addition of sodium chloride at the rate of five parts of salt, by weight, to one part of mercuric chloride induces penetration usually equal to that of the water carrying the

chemicals. The solution must contain 0.5 per cent of HgCl_2 in order to insure such penetration in Manor loam and in Leonardtown silt loam.

The penetration of copper sulphate alone in solution is extremely poor. Penetration is somewhat improved by adding sodium sulphate to the solution, and greatly improved by adding five parts of sodium chloride to one of copper sulphate. The penetration secured was markedly less than that of the water of the solution even with solutions containing 2 per cent of copper sulphate.

The penetration of kerosene is variable, increasing remarkably with increases in the percentage of moisture in the soil. Kerosene was used undiluted.

The mercuric-chloride plus sodium-chloride solution was used in most experiments; the penetrations secured with applications of 1 gallon per square foot were as follows:

In potting soil, penetration, as shown by chemical tests, was erratic, probably owing largely to differences in the chemical makeup of different lots of soil.

In Manor loam, penetration gradually increased from about 7 to 8 inches in soil containing less than 5 per cent of moisture, to 8 to 9 inches in soil with 17 to 19 per cent of moisture, and then decreased slightly in soil with 20 to 23 per cent of moisture.

In Leonardtown silt loam, penetration gradually increased from 6 to 8 inches in soil containing less than 5 per cent of moisture, to 8 to 9 inches in soil containing 15 to 20 per cent of moisture, and to 11 to 12 inches in soil containing 43 per cent of moisture.

The effect of soil moisture on penetration is not the same for all chemicals. In Leonardtown silt loam, 1 gallon of $\text{HgCl}_2 + \text{NaCl}$ solution and 1 gallon of kerosene penetrated to about the same depth in soil containing 1.8 per cent of moisture. In soil containing 19 per cent of moisture, 1 pint of kerosene penetrated as deeply as 1 gallon of $\text{HgCl}_2 + \text{NaCl}$ solution.

With solutions of $\text{HgCl}_2 + \text{NaCl}$, the penetration obtained by applying 1 gallon of solution is less satisfactory than that obtained by applying the same amount of chemicals in $\frac{1}{2}$ gallon of solution followed by $\frac{1}{2}$ gallon of water. Other proportions of solution to water gave similar results. The actual depth of penetration was about the same, but the distribution of the chemical was more uniform and the tests at points near the line of maximum penetration were markedly stronger where water followed the solution.

The data obtained will be of assistance in planning field treatments and make it possible to determine in the laboratory the strength and amount of solution required for the effective treatment of a soil, taking advantage of favorable soil moisture or of rainfall and of cheaper methods.

PART IV. POTATO GROWTH FOLLOWING CERTAIN CHEMICAL SOIL TREATMENTS

As stated in Part I, many of the chemical treatments tested as possible means of exterminating potato wart resulted in more or less severe retardation of growth of potatoes. A summary of the growth of potatoes in the treated plots during three seasons subsequent to the treatments is given below. The data here recorded are for relatively strong chemicals used in large quantities. Weaker appli-

cations made in the earlier chemical treatments gave very erratic results, the minute quantities of chemical breaking down, leaching, or forming new combinations in the soil. This is due in part, as are the fluctuations in the 100 treatments made at Sandy Run in 1921 and here summarized, to the nature of the available gardens. In no case has the soil been uniform throughout a garden, but has varied in composition by reason of mixtures due to stripping and coal-mining operations, addition of soil from the woods, the dumping of ashes and garbage, and unequal applications of manure. By distributing the treatments among these variable areas,¹⁴ however, it is believed that the results more truly represent the comparative effects of the various chemicals used, even though they are less uniform than those obtainable by selecting contiguous beds for several applications of like chemical. Prior to the use of chemicals, and again before planting each of the two following seasons, the ground was thoroughly spaded to the depth of 8 inches.

Soil treatments were applied dry or in solution as indicated in Table XXI. The chemicals applied dry were powdered to permit even distribution. Watering cans with rose tops were used for applying liquids. Two methods of application were used; either addition to the surface of the area treated, or mixing with the soil to a depth of 8 inches by spading.

Planting followed treatment after 10 days or less the first year, while no further treatment other than spading and cultivation was given the second and third years.

The tolerance of the potato plant for the chemicals used is expressed by the symbols indicated in the footnote of Table XXI. The stand, the growth of tops, and the yield of tubers are all taken into account in determining the rating. The data presented are for particular soils and conditions and no attempt is made to draw general conclusions from them. It is of interest to note that of these chemicals Bordeaux (pl. 1, D) in whatever concentration or amount tried, potassium permanganate in its higher dilution, and a proprietary compound called Qua-sul apparently were not injurious to growth in any year.

Carbolic acid and the weaker treatment with chloride of lime (pl. 1, A) showed poor growth or none in the first year and practically normal growth in the second and third. The heavier applications of chloride of lime exhibited residual toxicity the second year. Treatments with sulphur produced the reverse condition. During the first season the plots which had received 0.12 pound of sulphur per square foot made a normal growth of tops, while the 0.25 and 0.3 pound plots made poor and very poor growth, respectively. The tops died later in the season because of the increased soil acidity. A considerable number of tubers had formed, but owing to the death of the tops these remained very small. In the second and third seasons, except for a few burned sprouts in the weakest treatment, all plants were killed (pl. 2, C). In the process of oxidation the sulphur had increased the soil acidity beyond the limit of tolerance of the potato plants.

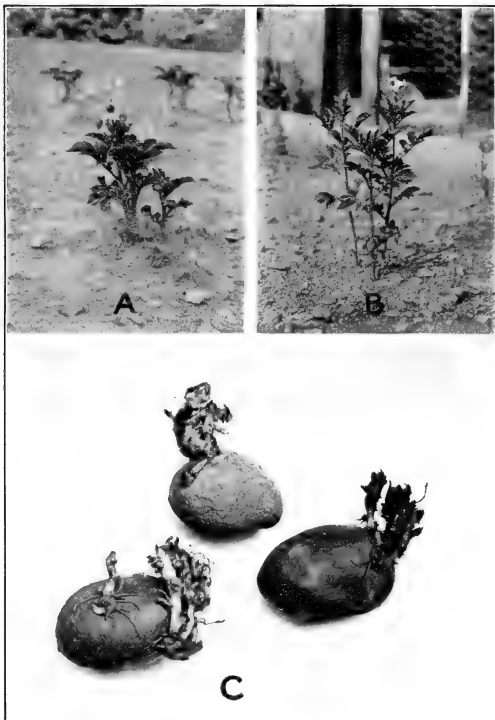
Copper sulphate (pl. 1, D), lime-sulphur (pl. 2, A), sodium carbonate, sodium chromate, sodium fluoride, a commercial weed killer, and the stronger cleaning solution treatments showed no growth in

¹⁴ Figure 3 shows the distribution of the plots.

TABLE XXI.—Summary of 100 chemical applications made near Freeland, Pa., 1921

Chemicals used	Treatment applied	Amount per square foot	How applied ^a	Number of plots treated	Growth ^b		
					1921	1922	1923
Borax.....	Dry.....	0.5 to 1 pound.....	S and M	5	O	X to XX	XX+
Bordeaux.....	{1 pound to 1 pound to 3 or 6 gallons	1 to 2 quarts.....	S and M	4	XXX	XXX	XXX
Carbolic acid.....	{Woburn.....	1 to 2 gallons.....	M	4	XXX	XXX	XXX
Chloride lime.....	{5 per cent.....	0.5 to 1 gallon.....	S	4	O	XXX	XXX
	{Dry.....	0.5 pound.....	S and M	2	O	XXX	XXX
	{Dry.....	1.6 pounds.....	M	1	O	X	XXX
Cleaning solution.....		{2 gallons.....	S	1	O	O	O
		{1 gallon.....	S	1	O	XX	XX
Copper sulphate.....	Dry.....	1 gallon.....	M	2	O	(X)	O to X
	{15 per cent commercial	0.5 to 1 pound.....	M	4	O	(X)	O to X
Formaldehyde.....	{10 per cent commercial	2 gallons.....	S	1	(X)	XXX	XXX
	{5 per cent commercial	2 gallons.....	S	2	X	XXX	XXX
Javelle water.....		2 gallons.....	S	2	XX	XXX	XXX
Kerosene.....		1 gallon.....	M	4	(X)	XX to XXX	XXX
Kerosene and crude oil.....		0.8 to 2 gallons.....	S	4	O	X to XX	XXX
	1 gallon+1 gallon.....	2 gallons.....	S	1	O	XX	XXX
Lime sulphur.....	{1 in 3 (gallons).....	0.5 gallon.....	S and M	2	O	(X)	X-
	{1 in 6 (gallons).....	0.5 gallon.....	S and M	2	O	X	X
	{1 in 12 (gallons).....	1 gallon.....	S	1	O	X	X
	{2 pounds to 25 gallons	0.5 gallon.....	S	2	O	X	X
Mercuric chloride.....	{2 pounds to 25 gallons	0.5 gallon.....	S	3	O	X to XX	X to XX-
Mercuric chloride and salt.....	{Dry.....	20 grams.....	M	3	XX	XX	X to XXX
Mercuric chloride and salt.....	1 pound to 6.25 pounds to 12.5 gallons.....	1 gallon.....	S	4	O	X	X to XXX
Oil, crude.....	1 pound to 25 pounds to 25 gallons.....	0.5 to 1 gallon.....	S	2	O	X	XXX
Potassium carbonate.....		0.5 gallon.....	S	2	O	X	XXX
Potassium permanganate.....	Dry.....	0.4 pound.....	M	1	O	X to XX	X to XXX
Pyroligneous acid.....	1 to 1,000.....	1 to 2 gallons.....	S	4	XXX	XXX	XXX
Qua-sul.....	1 in 50 or 100.....	0.6 gallon.....	S	2	X	XX to XXX	XXX
Sodium carbonate.....	Dry.....	0.3 gallon.....	S	2	XXX	XXX	XXX
Sodium chloride.....		{3 pounds.....	M	2	O	(X)	X to XX
Sodium chromate.....	Dry.....	12 pounds.....	M	2	O	(X)	X
Sodium fluoride.....	1 pound in 2 gallons.....	0.5 to 1 pound.....	M	4	O	O to XXX	XXX-
Sulphur, flowers of.....	Dry.....	0.4 to 1 pound.....	S	1	O	(X)	XX
Sulphur, inoculated.....	Dry.....	{0.25 pound.....	S and M	4	O	O	O
Weed killer.....	{0.12 pound.....	0.3 pound.....	S and M	2	XXX	O to (X)	X
Control plots.....	Dry.....	1 gallon.....	M	1	(X)	O	O to X
	10 or 20 per cent.....	Untreated.....	M	2	O	XXX	XXX
				5	XXX	XXX	XXX

^a M = Mixed with the soil by spading. S = Surface application. ^b O = No growth; (X) = growth barely possible; X = poor growth XX = fair growth; XXX = normal growth.



TREATMENTS GIVEN IN 1921 -PHOTOGRAPHS MADE IN 1922

A. Stunted growth of potatoes during 1922 in a plot treated before planting time in 1921 with lime-sulphur 1 to 5, one-half gallon per square foot. (Photographed August 10, 1922)

B. Spindling growth of potatoes during 1922 in a plot treated before planting time in 1921 with 1 per cent $HgCl_2$, 1 gallon per square foot. (Photographed August 10, 1922)

C. Growth of potatoes during 1922 in a plot treated before planting time in 1921 with 2 ounces of sulphur per square foot. (Photographed September, 1922.) The potatoes planted in the spring of 1922 failed to grow and the plot was replanted during the summer. In some cases the distorted stems did not reach the surface of the ground and few stems exceeded 1 inch in height

the first and practically none in the second and third seasons, except that the sodium chromate plot showed fair growth the third season. None of the plots treated with sodium chloride or borax produced growth during the first year. In the second season the five borax plots exhibited from poor to fair growth and in the third season exhibited fair to normal growth. Results with sodium chloride were more variable, the four plots ranging from no growth to good growth, and with no apparent cause other than that differences in soil types or drainage may have been the determining factor. Plots treated with these chemicals showed subnormal growth the third year. Pyroligneous acid, though showing much injury the first season and little the second, was highly variable in its effect on growth. During the third year normal growth occurred.

Mercuric chloride alone, and in combination with salt, except in the dry applications, killed the potatoes the first season and showed considerable toxicity the following year, both in the amount of growth which occurred and in the spindling character of plants (pl. 1, B, pl. 2, B). Growth during the third season was poor in the two plots treated with a 1 per cent solution of mercuric chloride at the rate of 1 gallon per square foot and in one of the plots treated with one-half gallon of the same solution, but was practically normal in the remaining plots.

Kerosene, crude oil, and a mixture of the two inhibited growth the first season, but permitted fair growth the second. The third season growth was poor in one of the plots treated with crude oil and normal in the other plots.

Formaldehyde showed injury during the first season (pl. 1, B), proportional to the strength of the application, and uniformly good growth for all plots the following two seasons.

All applications of the sulphuric acid and sodium chromate cleaning solution, familiar in the laboratory, used at full strength, killed the first plantings, but were sufficiently neutralized in the soil to allow some growth the following two years in two of the plots.

Javelle water practically prevented growth the first season, but fair to normal plants were produced the second and third.

When heavy chemical treatments are applied to sterilize soil, their toxicity, their alteration of the mechanical condition, and the changes which they may produce in their reaction may be injurious or not in variable degree. While it is true that as regards the growth of the potato plant the treated soil in many cases tends to return to the normal condition the second year, the reverse is sometimes the case, greater injury being evident the second season.

Therefore in applying a chemical for partial sterilization of the soil, the substance used, the rate of application, and the particular soil and climatic conditions which prevail where it is applied deserve very careful consideration from the standpoint of possible injury to plant growth as well as of its efficacy as a soil disinfectant.

(A summary is given at the end of each of Parts I, II, and III, pp. 320-321, 328-9, and 357-8).

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THE NUTRITIVE VALUE OF WHEAT: I.—EFFECT OF VARIATION OF SODIUM IN A WHEAT RATION¹

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INTRODUCTION

The classical experiments conducted by the Wisconsin Experiment Station (11) with heifer calves restricted to a ration obtained entirely from the wheat plant and salt which resulted in rough-coated animals of small girth and rough in appearance as contrasted with heifers receiving a ration derived solely from the corn plant which resulted in animals in fine condition, was considered by the writers as worthy of further investigation. In a region such as eastern Washington this is especially true, for the growing of wheat predominates there and the wheat plant in many cases comprises at least the bulk of the ration for stock.

From conversations with some of the pioneers of the State of Washington who grew wheat exclusively in the early days, the writers have learned that cattle and their offspring showed no ill effects which were regarded as due to rations that were restricted to the wheat plant. This also suggested a further study with wheat. The possibility that wheat grown in the Middle West might be different in composition from wheat grown in the Pacific Coast States seemed another good reason for further investigation. It was also considered that the wheat plant might have a different effect on livestock when consumed in pasturing than when consumed as mature grain and straw.

In 1919 work was started by the senior writer to try to determine if possible why the Wisconsin Experiment Station and the practical feeders of the Northwest obtained different results. In 1921 the junior writer took charge of the work and started a project on the nutritive value of wheat and wheat products. This was designed to study the nutritive deficiencies of wheat, limits in the use of deficient food elements, variation in the nutritive value of wheat and the factors on which variation depends, relative nutritive value of different varieties of wheat, and the nutritive value of the flour from the wheats studied. This paper is a progress report on a part of the work done.

REVIEW OF LITERATURE

According to Hart, McCollum, and collaborators of the Wisconsin Experiment Station (11),³ a ration restricted to the wheat plant did not seem to allow successful growth and reproduction. In a later publication (13), they expressed the opinion that the inferior results with the wheat-plant ration were probably due to mineral deficiency, and inherent toxicity in the wheat kernel, with possibly a third deficiency—vitamin A.

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³ Reference is made by number (italic) to "Literature cited," p. 374.

After some experiments on feeding swine a large amount of wheat, Hart, Miller, and McCollum (12) state that toxicity follows even in the presence of all the recognized factors for growth. Apparently in a like manner, wheat has seemed to others (3) to cause injury to the progeny of rats.

It has long been known that cereals in general, and wheat in particular, contain inadequate amounts of the elements sodium, chlorine, and calcium, when compared with the quantity contained in milk (Tables I and II). In feeding animals there is a greater probability of feeding insufficient sodium than potassium, which is in predominant quantity among the minerals in the cereals (27).

TABLE I.—*Mineral constituents in cereals and roughage*

Kind of cereal	Per cent, dry basis				
	Ash	K	Na	Ca	Cl
Wheat (4).....	1.866	0.59	0.035	0.056	0.095
Wheat (27).....		.515	.0106	.044	.088
Wheat (27).....		.431	.051	.044	.080
Corn (4).....	1.410	.396	.030	.014	.073
Oats (4).....	3.709	.460	.184	.112	.077
Wheat flour (4).....	.192	.058	.127	.022	.081
Wheat flour (27).....		.146	.069	.026	.076
Wheat straw (4).....	3.65	.842	.237	.217	.209
Corn stover (4).....	7.007	1.847	.065	.507	.308
Alfalfa hay (4).....	6.89	.832	.489	1.130	.161

TABLE II.—*Mineral constituents in milk (2), (17)*

Kind of milk	Per cent, dry basis (calculated)				
	Ash	K	Na	Ca	Cl
Cow.....	5.75	1.22	0.59	0.98	1.13
Goat.....	5.45	.76	.35	.99	.72
Human.....	1.68	.56	1.60	.29	.39
Sheep.....	4.39	4.23	.33	.91	.68
Horse.....	4.34	.94	.11	.97	.34

The mineral analyses of the various parts of the body show that potassium predominates in the muscles, brain, liver, saliva, and milk; and sodium in blood, serum, lymph, pancreatic fluid, bile, and gastric juices. Bone is higher in sodium than in potassium.

The amount of sodium in the ash of wheat is reported from a trace to a fraction more than 7 per cent. In the writers' analyses the sodium is found to exceed 12.5 per cent of the total ash. The amount of potassium is comparable with the results reported by other investigators (15). The percentage of calcium is about the same and the chlorine less in the wheat analyzed and used by the writers.

The percentage range of the minerals in wheat may be summarized as follows: Iron and aluminum, 0.27 to 0.40; potassium, 0.34 to 0.49; magnesium, 0.075 to 0.14; calcium, 0.38 to 0.63; phosphorus, 0.36 to 0.47; sulphur, 0.036 to 0.083; and sodium, a trace to 0.23.

In many feeding trials calcium has been included, and sodium chloride has been given *ad libitum*; or salt mixtures have been pre-

pared with varying proportions of different kinds of salts, among which are salts containing calcium, sodium, and chlorine (19, 20, 23, 24). However, the inclusion of these elements with the restricted wheat ration and vitamin A, or wheat in combination with wheat gluten and vitamin A, have not met the needs of the mother rats in caring for their young in the Wisconsin experiments.

In the following pages data will be presented showing that rats can be successfully fed rations similar to those used at Wisconsin, and that through certain adjustments in making up the mineral deficiencies these rats can properly care for their young and successive generations may be brought to maturity. The effect of the variation of the amount of sodium in such a wheat ration is also shown.

With the exception of calcium and phosphorus (5), comparatively little work seems to have been done to determine the optimum amounts of the various inorganic elements which are needed by animals under varying conditions. Even the work on calcium and phosphorus is far from complete. Forbes (2, p. 145), in 1909, made the following statement:

Our knowledge of the amounts and kinds of mineral matter required by animals is indefinite and fragmentary. Much progress has yet to be made in this field. Such recommendations as we are able to make should be regarded only as general indications of the truth.

Forbes has since made an extensive study of phosphorus (5) and calcium, as reported in Ohio Agricultural Experiment Station bulletins (6, 7, 8, 9, 10, 25, 26) and in other papers. Sherman (27) also has recognized the need of a further quantitative study of the inorganic elements.

Emphasis has been placed upon the acid-base balance (27, 3) of inorganic elements. Later information (15, 16) tends to show that excess of mineral acids is not as harmful as was formerly believed. Lamb and Evvard (16) make this statement: "If the other elements in a natural ration are satisfactory, it is not necessary to balance the acid and basic mineral elements for growing swine."

That there is lack of definite information regarding the inorganic elements in human and animal nutrition may be shown by an examination of such texts as those by Lusk (18), Henry and Morrison (14), and Armsby (1).

Harnemann, quoted in Lusk, states that in man the sodium-chloride balance was maintained with 5 gms. of salt daily, which was half to one-quarter the amount usually taken. Lusk states that "one of the most important questions of the time concerns the determination of the quantity of salts in the food necessary to prevent malnutrition in children."

Henry and Morrison state in 1922 (14) that "at present there is little data regarding the minimum amounts of lime and phosphorus which will permit normal development of growing animals." They say that a reasonable allowance of salt for a horse is 2 oz. per day; and that milk cows should receive at least 1 oz. a day; that 0.75 oz. a day per 1,000 lbs. of live weight with 0.3 oz. in addition for every 10 lbs. of milk produced is generally sufficient. "A plan followed by many dairymen is to mix 0.5 lb. to 1 lb. of salt with each 100 lbs. of concentrates, and then in addition to provide salt so the cows can have access to it and take all they wish."

In trials at the Iowa Experiment Station during five years, Evvard found that 2-year-old steers fattened on typical Corn Belt rations, including corn silage, and allowed free access to block salt, consumed just about one-third ounce of salt per head daily. The gains of the animals were very satisfactory. "In a trial carried on by the Kansas station it was found that during the summer yearling and 2-year-old steers on pasture licked about 1 oz. of salt per head daily from salt blocks placed in the pasture" (14). "In an experiment in France, sheep fed 0.5 oz. of salt daily with their feed gained materially faster than those fed no salt, and also somewhat more rapidly than others fed 0.75 oz. daily. The fleeces of the salt-fed sheep were of better quality and heavier than those of the sheep fed no salt" (14).

Armsby emphasizes the need of further study of the inorganic elements. He feels that the amount of these elements actually necessary is less than is often supposed. The data which he gives regarding sodium, potassium, and chlorine, seem to be conflicting.

An investigator in 1907 (21) stated that the ability of the body to take up sodium salts is practically unlimited. He speaks of 12 to 15 gms. of sodium chloride as the normal amount used by man, and 7 to 8 gms. as the normal amount on a milk diet.

EXPERIMENTAL DATA

In the writers' preliminary experiment 9 young white rats were divided into 3 lots and fed the same basal ration, consisting of 100 gms. of ground wheat, 10 gms. of wheat gluten, and 5 gms. of prepared butterfat. The ration contained approximately 16.6 per cent of protein and should satisfy the requirements of the white rat as far as protein, fat, carbohydrate, and vitamins are concerned. The food given to lot No. 1 consisted of the basal ration plus 0.5 gm. sodium chloride. For the second lot 0.5 gm. calcium chloride was added to the basal ration, and for the third lot, a combination of 0.5 gm. sodium chloride and 0.5 gm. calcium chloride was given with the basal ration.

Two of the rats given the rations containing the sodium chloride died at 4 and 5 weeks respectively, while the remaining one developed slowly but very unsatisfactorily. Likewise, two of the rats given the calcium chloride and sodium chloride combination died at 3 and 11 weeks, and the one remaining made somewhat more rapid development than the rats given the sodium chloride. The rats fed the calcium-chloride and sodium-chloride combination seemed to withstand approximately double the quantity of chlorine present in the sodium-chloride ration. The lot receiving the calcium-chloride ration not only made better gains than was noted in case of either of the other two lots, but also without mortality.

The female in the calcium-chloride lot finally raised six young rats successfully. Toward the end of the nursing period, the mother showed signs of becoming blind, and her head was twisted to one side. She conceived again but aborted. In the third gestation period much difficulty was experienced at parturition. At this time there was no doubt as to the effect produced on the nervous system, and blindness was apparent. The mineral content of the ration for this rat was then changed to 0.1 gm. sodium chloride with 0.4 gm. calcium chloride, when she again successfully reared two litters of seven and four young, respectively.

The first young rats from this calcium-chloride mother, and two young stock rats, were divided into two lots, one of which (lot A) received the calcium-chloride ration, and the other (lot B) received the 0.1 gm. sodium chloride and 0.4 gm. calcium-chloride ration. In this plan it will be seen that the probability of the transmission of any beneficial stabilizing influence which might have originally been present through the use of other foodstuffs was greatly reduced.

In the remaining six rations of this experiment 0.5 gm. of calcium chloride and a varying amount of sodium in the form of sodium bicarbonate were added to the basal ration as follows: Per cent, Na: (A), 0.228; (B), 0.260; (C), 0.287; (D), 0.354; (E), 0.525; (F) 0.785; (G), 1.29; and (H), 2.58. The rations contained 0.21 per cent calcium and 0.27 per cent chlorine. In all other respects the rations were alike.

The wheat was finely ground. The gluten used had the qualities specified by the patent taken out by one of the writers (22). The butterfat was carefully prepared by filtering out the casein and washing out the salts.

Figure 1 gives the composite growth curves for the eight different rations.

The curve for ration A is the average of four closely agreeing curves and it will be noted that it is decidedly flat in comparison with the normal growth curve. There was no successful production of young on this ration and apparently only one conception.

The curve for ration B shows much better growth than for A, and in fact follows the normal-growth curve for more than four weeks. Young were successfully produced on this ration, and they in turn produced a third generation.

For ration C the curve shows better growth than for B, as it quite closely approaches the normal-growth curve for 16 weeks. One vigorous litter of young was produced and later a second litter by the same mother. Another female produced seven young which were all lost. The first litter mentioned produced a third generation.

The curve for ration D is practically identical with that for C for 10 weeks. Three litters of young were born within a week and a fourth shortly after. Three second litters were produced later.

The curve for E shows that this ration produced the best growth of any of the rations in this series. Vigorous young were produced on this ration.

The F curve shows a less rapid gain than the preceding rations, although the gains were the most economical, as noted elsewhere. One satisfactory litter of young was produced.

The G curve is quite similar to the F curve but no young were produced.

The curve for ration H is flat, although it is somewhat better than for A, at least up to six months. One litter of young was produced after the point at which the curve ends, when the mother was about a year old. One of these young died at 2 months and one at 3½ months, the latter weighing 32 gms. Two were alive at 5 months, one weighing 103 gms. and one 56 gms.

Figures 2, 3, 4, 5, and 6 show the composite curves for males and for females on rations A to E, respectively. In the remaining rations the two curves (not shown) are practically identical.

After the completion of the work just discussed, three of the rations, A, D, and H, were repeated with a different lot of wheat, and results supporting those just given were obtained.

The amount of feed in grams per gram of gain for the first 4½ months, and for the first 9 months is as follows:

	A	B	C	D	E	F	G	H
4½ months.....	40.7	12.2	11.7	9.9	8.7	7.2	10.0	16.1
9 months.....	38.1	21.7	21.1	20.5	-----	14.1	16.9	22.6

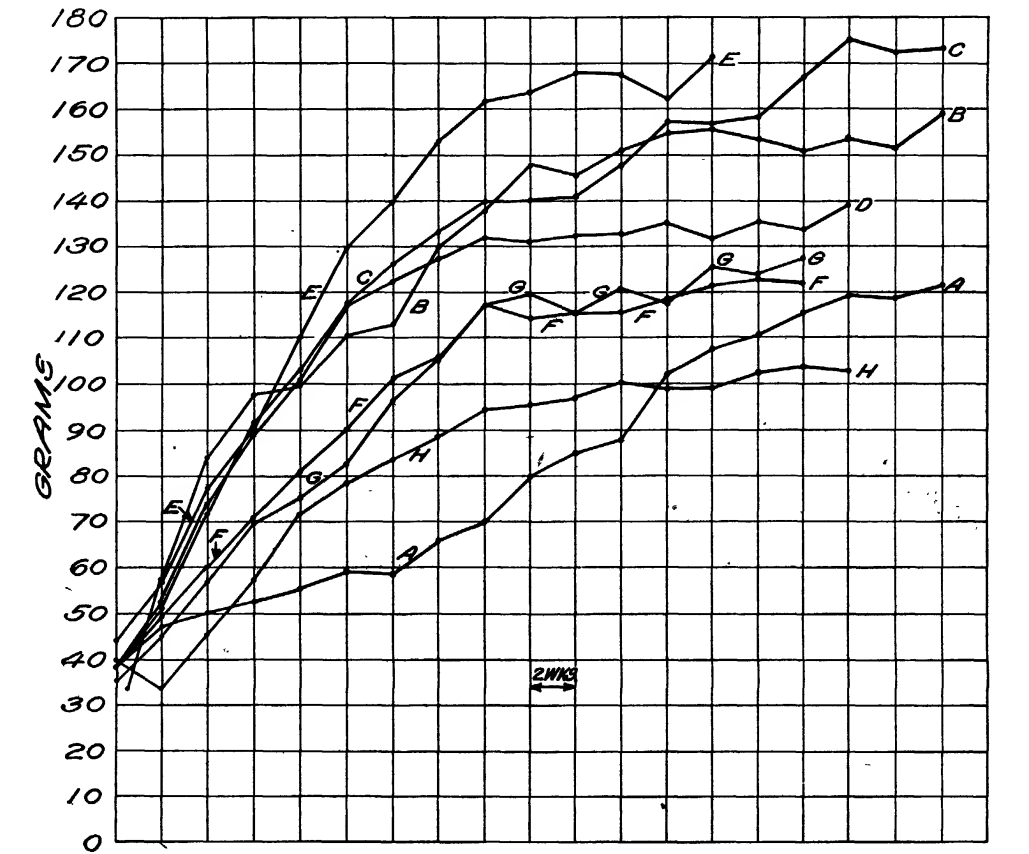


FIG. 1.—Composite growth curves for rats fed on the eight different rations

It will be noted that the addition of a small amount of sodium to this ration greatly increased the economy in the use of the feed. Increase of the amount of sodium fed seemed to effect a greater economy, up to a certain point. But, beyond this point, additional sodium seemed to reduce the economy. Ration F containing 0.785 per cent sodium seemed to produce the most economical gains in weight. However, this amount did not seem to promote the most successful reproduction.

It is also interesting to note the amount in grams of sodium required per gram of gain:

	A	B	C	D	E	F	G	H
4½ months.....	0.0928	0.0317	0.0336	0.0350	0.0457	0.0565	0.129	0.4154
9 months.....	.0869	.0564	.0606	.0726	-----	.1107	.218	.5831

The daily consumption of feed in grams per rat was the largest in lot B, with decreasing requirements for lots C, D, and the other rations, as indicated in the following tabulation:

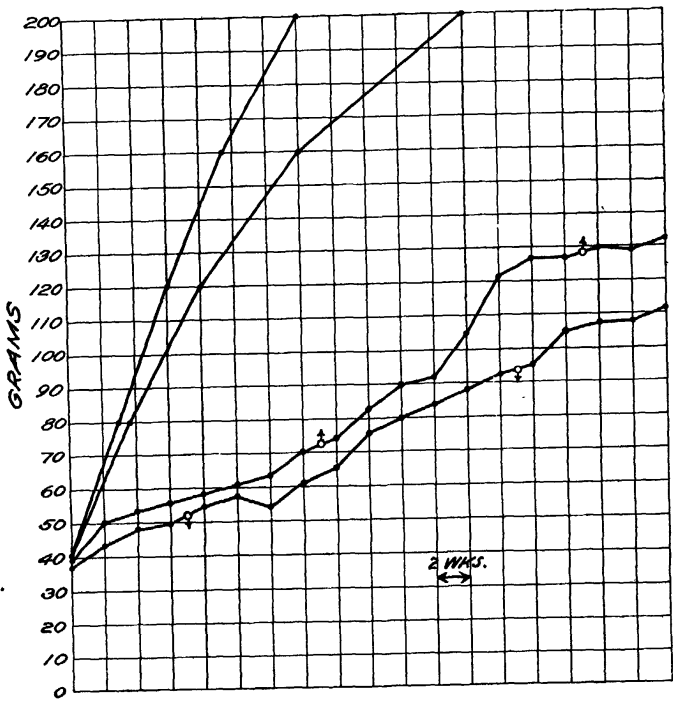


FIG. 2.—Composite growth curves for male and female rats fed on ration A. Normal growth curves also shown

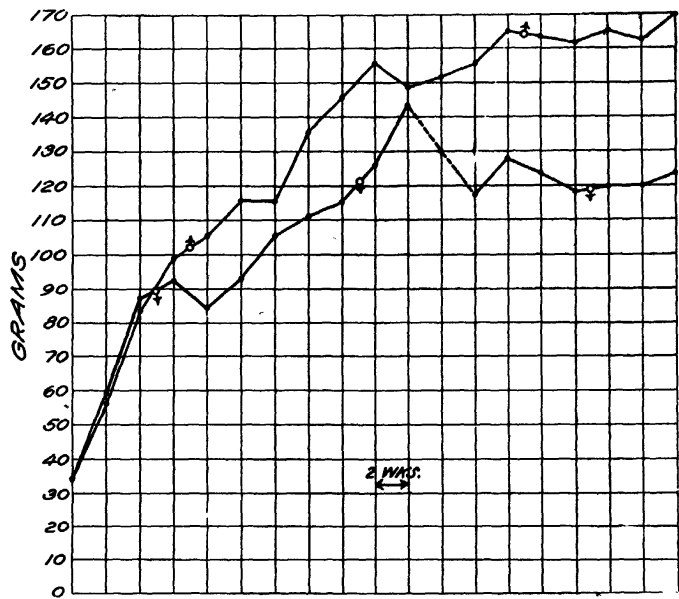


FIG. 3.—Composite growth curves for male and female rats fed on ration B

	A	B	C	D	E	F	G	H
4½ months.....	12.13	13.85	11.59	7.75	8.07	5.10	5.60	5.67
9 months.....	13.04	15.41	14.02	9.94		7.04	5.82	7.51

A corresponding order of course prevails for the protein, butterfat, calcium, and chlorine intake. The amount of sodium in grams consumed by each rat each day is as follows:

	A	B	C	D	E	F	G	H
4½ months.....	0.0276	0.036	0.033	0.037	0.042	0.040	0.072	0.172
9 months.....	.0297	.040	.040	.035	-----	.055	.075	.194

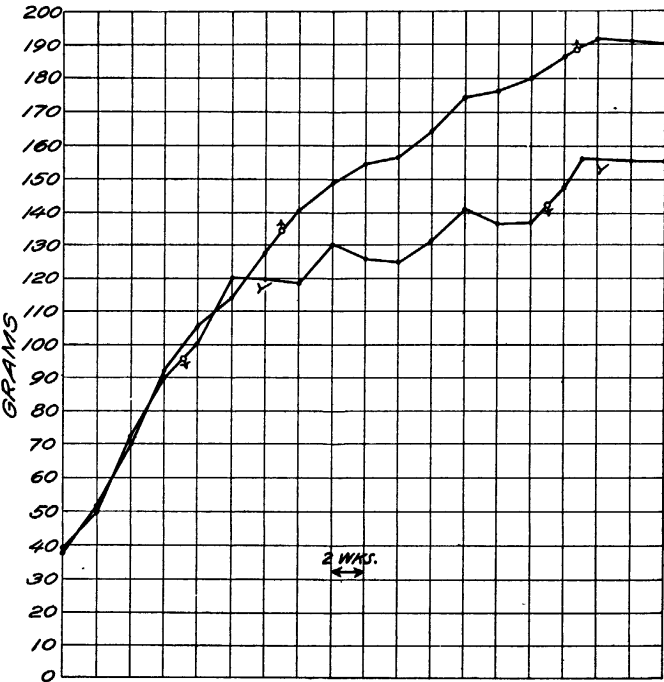


FIG. 4.—Composite growth curves for male and female rats fed on ration C

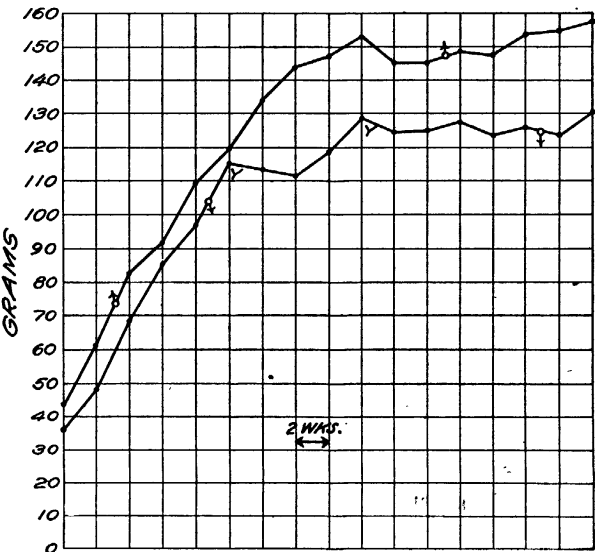


FIG. 5.—Composite growth curves for male and female rats fed on ration D

From this it would appear that approximately 40 mgms. of sodium per day is an optimum amount, in such a ration. Twelve or thirteen gms. of feed per day in ration A did not furnish that much sodium,

while 14 or 15 gms. of feed per day in B did supply that much, and much better growth and reproduction resulted. Smaller amounts of feed in rations C, D, E, and F furnished this amount of sodium, with greater economy in food consumption, there was better reproduction, and the gains were more rapid than was the case on ration A. The average daily gain in grams per rat for the two different periods is shown as follows:

	A	B	C	D	E	F	G	H
4½ months.....	0.30	1.13	0.99	0.78	0.82	0.70	0.56	0.42
9 months.....	.34	.7	.66	.48	-----	.50	.35	.33

With the larger amounts of sodium consumed per day in rations G and H, there was a lower average daily gain and a poorer economy of feed.

It is evident that the variations in growth were not due to the nature of the proteins or to lack of vitamin A or vitamin B—because the best gains resulted when the intakes of these essentials were less.

The animals on rations C and D seemed to have a tendency to produce young at an earlier age than those on the other rations. There was a marked group difference in the coats of the various lots. The hair was very fine in lot A, and coarser in lots B, C, and D. The coat of lot C was of excellent quality.

DISCUSSION

It has been found that rats not only maintain themselves but make some growth on wheat grown in the Pacific Northwest when this wheat is supplemented with gluten, butterfat, and calcium chloride. The addition of sodium seemed to make it possible for the rats to reproduce and successfully care for their young. The lot A rats were later transferred to the lot B ration, and 30 days later one of the females gave birth to four young.

Various combinations of mineral supplementation have been studied, and some of them have produced results similar to those reported by the Wisconsin Experiment Station. The altering of the mineral supplement has caused different degrees of improvement. Although the writers' work shows a higher sodium requirement than that shown by the work of some other investigators, it must be considered that the animals used in the present investigations were put on the various rations as soon as they were weaned and therefore

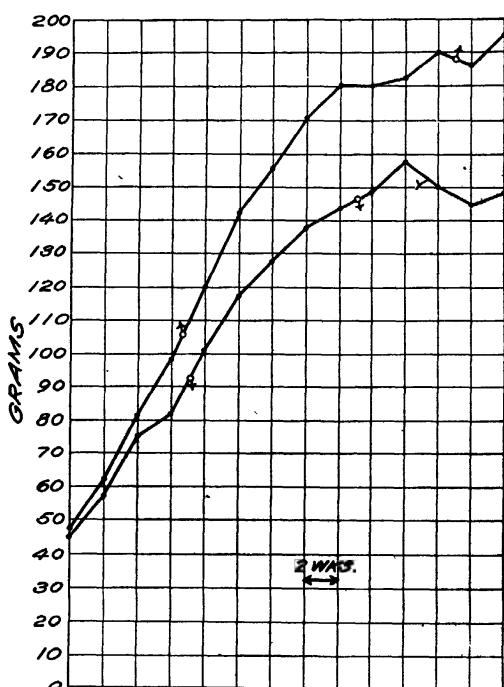


FIG. 6.—Composite growth curves for male and female rats fed on ration E

had less opportunity to benefit from any sodium which might be stored up by older animals. There is also no variation of other elements in these rations, and the reproduction records and the record of the total food requirement have been taken into consideration.

Analyses show marked differences in the mineral composition of wheat. These differences may have been partly responsible for the failure noted in the Wisconsin experiments. The success attained at the Washington Experiment Station following adjustments of minerals has pointed to the importance of ions rather than salts.

The success experienced in raising livestock on wheat rations on the farms of the Pacific Northwest has been believed to be due largely to the salts in the soils and water, and to the amount of mineral in the wheat. The opinion has been held that the potassium carbonate used with the Wisconsin wheat should have been replaced by sodium carbonate, or, at least, by sodium bicarbonate or some organic salt of sodium. On the basis of the results obtained here, it appears that the substitution of sodium for the potassium in the Wisconsin rations might have entirely altered the results there.

SUMMARY

A study has been made of the effect of a variation of sodium in a wheat ration on growth and reproduction. The ration containing no more sodium than the amount present in the wheat was not accompanied by normal growth or successful reproduction. The addition of sodium in the form of sodium bicarbonate was accompanied by much better growth and successful reproduction. Large amounts of sodium caused detrimental effects. From the results it appears that 0.23 per cent of sodium is too small an amount, 0.53 per cent is the most satisfactory when both growth and reproduction are considered, and that 0.785 per cent and amounts above this are detrimental.

It is also thought that a proper adjustment of the amount of sodium in the ration caused an economy in the use of feed. In the rations which proved the most successful, the animal consumed an amount of feed which furnished approximately 40 mgms. of sodium per animal.

Growth and reproduction were successful on a wheat ration which was corrected for certain nutritional deficiencies.

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AN APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES¹

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INTRODUCTION

In previous papers² one of the writers has described coefficients of inbreeding and relationship designed to make possible the interpretation of livestock breed histories in terms of the Mendelian theory of inbreeding and crossbreeding. The formula for the coefficient of inbreeding may be repeated here for convenience:

$$F_x = \Sigma[(\frac{1}{2})^{n+n^1+1}(1 + F_a)]$$

In this formula, F_x is the required coefficient, and F_a is the similar coefficient for any common ancestor that makes the closest connecting link between a line of ancestry tracing back from the sire and one tracing back from the dam. The generations from sire and dam to such a common ancestor are designated n and n^1 , respectively. The contribution of a particular tie between the pedigrees of sire and dam is $(\frac{1}{2})^{n+n^1+1}(1 + F_a)$. There is a factor $\frac{1}{2}$ for every generation in the tie between the germ cells which unite to form the individual, reckoning the germ cells as each half a generation from sire and dam. The factor $(1 + F_a)$ measures the effect of prepotency of a common ancestor that is himself inbred. The total coefficient is simply the sum of all such contributions. It is to be noted that the same animal may form the tie between many different pairs of ancestral lines of the sire and dam.

This coefficient was shown theoretically to give the coefficient of correlation between the egg and sperm which unite to produce the individual in question. As far as Mendelian factors are involved, its use implies the definition of inbreeding as the bringing together of similar germ cells. It was also shown that this coefficient measures the percentage reduction from the average degree of heterozygosis in the foundation stock. As experiments with different kinds of animals and plants have indicated that the effects of inbreeding, such as decline in vigor, fixation of type, prepotency in crosses, vary directly with the increase in homozygosis, the coefficient appears to be the proper one to use from the physiological standpoint.

It may be well to call attention to the point that the coefficient is not an absolute, but a relative, measure of a quality of an animal. It measures the probable similarity of the germ cells which united to produce him, relative to the similarity of random germ cells from the foundation stock. Random-bred descendants of the foundation stock would have a coefficient of zero, even though the foundation stock itself might be highly inbred relative to more remote ancestors.

¹ Received for publication Oct. 7, 1924; issued September, 1925.

² WRIGHT, S. COEFFICIENTS OF INBREEDING AND RELATIONSHIP. *Amer. Nat.* 56: 330-338. 1922.

———. MENDELIAN ANALYSIS OF THE PURE BREEDS OF LIVESTOCK. I. THE MEASUREMENT OF INBREEDING AND RELATIONSHIP. *Jour. Heredity* 14: 339-348, illus. 1923.

Similarly, from the viewpoint of decrease in heterozygosis, the coefficient measures merely the change from the situation in the foundation stock toward perfect homozygosis, whether the foundation stock showed 50 per cent heterozygosis or 1 per cent or the infinitesimal amount which would doubtless be found with complete knowledge of the genetic constitution. In analyzing breed history, it is as legitimate to trace all pedigrees back to some arbitrary date as to trace them to the beginning of the herdbook. It is merely necessary to exercise due care in interpreting the results as relative to whatever foundation stock is used.

Closely related to the coefficient of inbreeding is the coefficient of relationship, which gives the degree of correlation to be expected between two individuals (X and Y) in characteristics which are entirely genetic and without dominance, conditions under which the correlation between parent and offspring or between brothers in a random-bred stock is $+0.50$. The interpretation, of course, depends on the choice of foundation stock.

$$R_{xy} = \frac{\sum \left[\left(\frac{1}{2} \right)^{n+n^1} (1 + F_a) \right]}{\sqrt{(1 + F_x) (1 + F_y)}}$$

In this formula F_x and F_y are the coefficients of inbreeding of the two individuals, F_a is that of the closest common ancestor connecting a pair of ancestral lines in their pedigrees, and n and n^1 are the number of generations from X and Y to this common ancestor along the lines in question.

APPLICATION TO BREED HISTORY

These coefficients have been applied, in a recent paper,³ to the analysis of Thomas Bates' methods of breeding in developing his famous Duchess family of Shorthorns. The coefficient of inbreeding of each of the 64 Duchesses, and of their sires and dams, was calculated from the pedigrees carried back to the foundation stock of the breed as completely as the pedigrees are recorded in the herdbook. The degrees of relationship of all of the Duchesses, and of their sires and dams, to the bull Favourite (252), the most important foundation animal of the breed, were also found from the complete pedigrees.

The Duchesses, however, came fairly early in Shorthorn history. The longest straight female line in the pedigrees was only 13 generations. Even here it was a rather tedious process to work out the coefficients for the later animals. It is obvious that the amount of work would become practically prohibitive in dealing with the breeds as they are to-day. The complete pedigree of a modern Shorthorn would require the tabulation of several million names. Fortunately, there is a very simple approximate method, the results of which can be brought as close as desired to the complete method.

This approximate method, which it is the purpose of the present paper to describe, rests on the tabulation of random samples of the pedigrees of sire and dam. The reliability of the results can be tested by the ordinary theory of sampling.

³ WRIGHT, S. MENDELIAN ANALYSIS OF THE PURE BREEDS OF LIVESTOCK. II. THE DUCHESS FAMILY OF SHORTHORNS AS BRED BY THOMAS BATES. *Jour. Heredity* 14: 405-422, illus. 1923.

It is necessary that the sample lines be chosen wholly at random. For obvious reasons, common ancestors are more likely to be males, in livestock breeding, than females. Thus straight male or straight female lines can not give a fair basis for calculating the number of ties between two pedigrees.

A system of alternating male and female lines has been tried out by the writers; and, while it gives fairly satisfactory results, the theory of sampling can not be applied in a simple way to calculate a probable error. If it could be calculated, the probable error would undoubtedly be somewhat larger than where truly random lines are used. With this or any other regular sequence of sires and dams the same lines tend to recur in different pedigrees and contribute more or less than would a random line, depending on whether important foundation animals happen to be represented in them or not.

A truly random line of ancestry can be obtained by letting the sequence of sires and dams which is to be traced back in the herdbook be that of the heads and tails, respectively, in a coin-tossing experiment. The same sequence should not, of course, be used frequently in the same study. The writers use a record of 1,000 coin tossings as material for obtaining random sequences. The sequence for each new line is begun where the last leaves off. A given sequence has little chance of repetition, since the number of generations in the lines varies.

The simplest possible sample which can show a connection between sire and dam is obtained by tracing back two ancestral lines, one on the sire's side and one on the dam's side. The scheme is illustrated in Tables I and II.

TABLE 1.—A two-line pedigree sample of the Shorthorn bull Millionaire (79438)

[An example of a case in which an ancestral connection is shown]

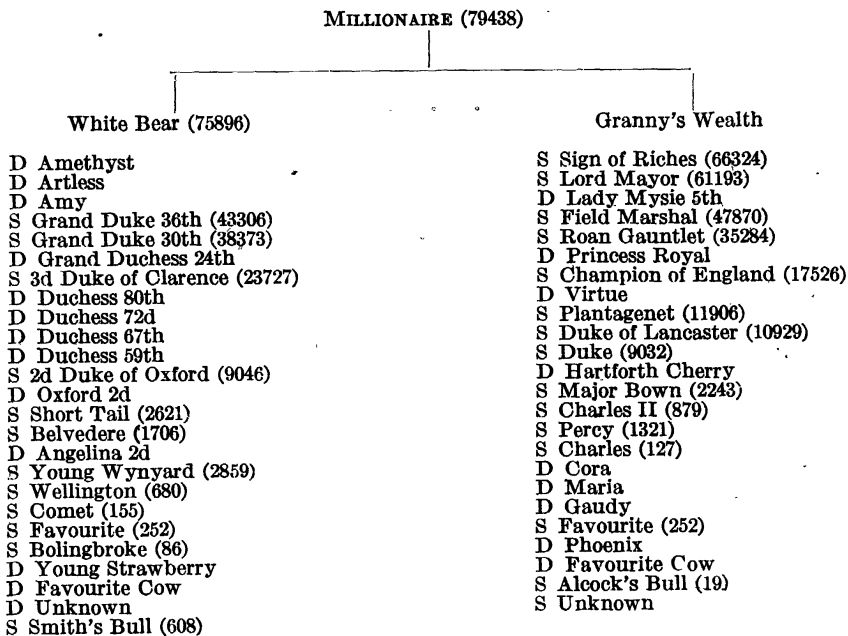


TABLE II.—A two-line pedigree sample of the Shorthorn bull Scottish Victor (79925)

[A case in which no ancestral connection is shown]

SCOTTISH VICTOR (79925)

Cairo (72151)	Stella 2d
S Prince of Fashion (64597)	S Matchmaker (69105)
D Primrose 3d	S Pride of the Morning (64546)
S Norseman (56233)	S Star of the Morning (58189)
D Nonpareil 20th	D Baronness 6th
S Cumberland (46144)	S Field Mashal (47870)
S Pride of the Isles (35072)	D Azalea
D Golden Days	S Caesar Augustus (25704)
S Lord Raglan (13244)	S Champion of England (17526)
D Brenda	D Virtue
S Maunby (7223)	S Plantagenet (11906)
S Clementi (3399)	D Madaline
D Cassandra	D Landlady
D Garland	D By Pilot (496)
S Matchem (2281)	S Pilot (496)
D By Farmer (251)	D Red Rose
S Farmer (251)	S By Punch (531)
S Bumper (101)	D By Broken Horn (95)
S Windsor (698)	S Broken Horn (95)
S Favourite (252)	D By Hubback (319)
D Phoenix	D By Bank's Bull
S Foljambe (283)	S Bank's Bull
D Haughton	
D By Colling's Bull	

In the case of the Shorthorn bull Millionaire (79438) (Table I), the sire (White Bear 75896) and dam (Granny's Wealth) are both recorded. Random sequences of S's and D's are then written in columns below each parent, extending sufficiently to include the foundation stock. The line of ancestry is then traced back in the herdbook, the sire being looked up where S occurs in the column and the dam for each D. In the case of White Bear (75896), the sequence happened to run DDDSSD . . . , etc. Thus White Bear's dam, Amethyst, is recorded, but not his sire. Then follow *her* dam, Artless, *her* dam, Amy, *her* sire, Grand Duke 36th (43306), and so on until the herdbook fails. A single random line of ancestry of Millionaire's dam is tabulated in the same way, the sequence in this happening to run SSDSSDS . . . Of course a second sample of the ancestry of the same animal would probably not show the same sequence of sires and dams, and it may be well to make clear that a single sample of this sort is of practically no value as an indication of the inbreeding of an individual. But the average obtained from a large number of such samples should not differ appreciably from the true value.

Two-column samples of this kind fall at once into two alternative categories; those which show an ancestral connection, as Millionaire (79438), and those which do not, as Scottish Victor (79925), Table II. In the latter case the coefficient is zero as far as the sample indicates. In the former case a contribution of $(\frac{1}{2})^{n+n^1+1}(1+F_a)$ is indicated if the common ancestor *A* is *n* generations back of the sire and *n*¹ back of the dam. The sire has 2^n ancestors in the *n*th generation and the dam 2^{n^1} in the *n*¹th generation. The sample pair of lines is thus only one among 2^{n+n^1} possible pairs going back as far as the common ancestor. If the single pair of lines is a fair sample of the total, its contribution must be multiplied by 2^{n+n^1} to obtain an estimate of the inbreeding of the whole pedigree. On carrying out this multiplication, *n* and *n*¹ disappear, and the coefficient takes the simple form $\frac{1}{2}(1+F_a)$. Thus, in calculating the

inbreeding indicated by a two-column pedigree, it is not necessary to count the generations to the closest common ancestor; it is merely necessary to note whether there is a tie, and what animal is responsible for it. Neglecting for the moment the effect of inbred common ancestry, the coefficient merely takes the values 50 per cent and 0 per cent under the two alternatives. As noted before, such a determination means practically nothing as far as the individual is concerned. But by determining the proportion of such ties in a sufficiently large random sample of a family or breed, a measure of the average degree of inbreeding of that family or breed can be obtained to as high a degree of accuracy as desired. If, for example, 40 two-column pedigrees show a common ancestor and 60 do not, an average inbreeding of 20 per cent ($=40 \times 0.50$) is indicated, again neglecting the term $(1 + F_a)$.

The probable error of the percentage of ties can be calculated by the usual formula, $E_p = 0.6745 \sqrt{\frac{pq}{N}}$, where N is the number of cases, p is the observed chance of occurrence of a tie, and $q (=1-p)$ is the chance of nonoccurrence. In the case cited, $E_p = 0.6745 \sqrt{\frac{0.40 \times 0.60}{100}} = 0.032$. As 40 per cent of ties corresponds to an inbreeding coefficient of 20 per cent, the probable error of the latter must be rated down proportionately, giving 20 ± 1.6 per cent. Allowance for the factor $(1 + F_a)$ will be considered later.

The method may be used to calculate the inbreeding coefficient of an individual, by finding the percentage of ties in a large number of random two-column samples from his pedigree. Increased accuracy may be obtained in this case, however, by a combination of the approximate method with the complete method. The pedigree may, for example, be tabulated completely for five generations, continuing each of the 32 lines to the foundation stock by the random method. As there are 16 random lines back of the sire and 16 back of the dam, there are 256 tabulated pairs of lines to be considered for possible ties. The total number of possible pairs of lines tracing n generations back of the sire and n^1 back of the dam is thus $\frac{2^{n+n^1}}{256}$ times the sample, if n and n^1 are both greater than four in this case. The contribution of an observed tie this distance back in the pedigree is $(\frac{1}{2})^{n+n^1+1} (1 + F_a)$. Multiplying by the number of pairs of lines which it represents gives $\frac{1}{512} (1 + F_a)$ as the contribution to be assigned the observed tie as a sample of the complete pedigree. This expression, it will be noted, is free from n and n^1 as in the two-column case. It is easy to see that, in general, the contribution to be assigned to a tie in the random part of a pedigree which is complete for k generations back of the parents is $(\frac{1}{2})^{2k+1} (1 + F_a)$.

If a common ancestor appears in the complete portion of the pedigree on one side and in the random portion on the other side, this formula requires modification. Letting n be less than k but n^1 greater, the ratio of possible to tabulated pairs of lines tracing n generations back of the sire and n^1 generations back of the dam is $\frac{2^{n+n^1}}{2^{n+k}} = \frac{2^{n^1}}{2^k}$, instead of $\frac{2^{n+n^1}}{2^{2k}}$, as in the case previously considered.

Multiplying by $(\frac{1}{2})^{n+n+1} (1 + F_a)$ gives $(\frac{1}{2})^{n+k+1} (1 + F_a)$ as the contribution to be assigned a tie in this case. A tie in the complete portion of the pedigree on both sides of course makes the unmodified contribution of $(\frac{1}{2})^{n+n+1} (1 + F_a)$. In dealing with partially complete pedigrees, care is of course necessary to see which pairs of random lines are eliminated from consideration by ties involving the complete part of the pedigree.

We are now in a position to consider more fully the method of calculating the average inbreeding of a group of animals. The ties between the sires and dams should be tabulated by the common ancestor involved. Certain of these animals may be found to be responsible for a large number of ties, others for only a few each. The pedigrees of those common ancestors which recur frequently should then be tabulated completely for a considerable number of generations in order to obtain a good estimate of their individual inbreeding by the method just discussed. For those which recur infrequently it is usually sufficient to assume an average degree of inbreeding equal to that of the breed as a whole at about the same time. The values adopted for the inbreeding of the various common ancestors (F_a) can then be used in the term $(1 + F_a)$ in calculating the total coefficient. For example, the closest connection between the sire and dam of the bull Millionaire (79438), in Table I, is the bull Favourite (252). Favourite happens to be responsible for many more ties in Shorthorn pedigrees than is any other animal. As he came early in the history of the breed, his complete coefficient of inbreeding can be calculated without difficulty, and it turns out to be 19.2 per cent. Millionaire (79438) thus must be assigned a weight of $0.50 (1 + 0.192) = 0.596$ in finding the average degree of inbreeding of any tabulation of Shorthorns in which he is included.

The probable error must be rated up to the final value. If p is the proportion of ties observed, q ($=1-p$) the proportion of pairs of lines which do not contain ties, and N is the number of pairs of lines

compared, the probable error of F_x is $0.6745 \sqrt{\frac{pq}{N}} \times \frac{F_x}{p}$ to a first approximation. For two-column pedigrees, N is of course simply the number of animals chosen from the breed. If four-column pedigrees are used, there are four possible ties in each pedigree, so that N is four times the number of animals. With eight-column pedigrees, N is 16 times the number of animals, etc. The probable error is reduced somewhat if partially complete pedigrees are used instead of ones in which all lines back of sire and dam respectively are purely random. There is no element of chance in the ties in the complete portion of such pedigrees, so that the contribution of those ties has no probable error. In applying the formula to partially complete pedigrees, p should include only ties involving random lines, and its probable error should be multiplied by the ratio of the portion of F_x , due to such ties to p .

These formulae give the probable error of the approximate formula for the particular animals chosen. Strictly, the probable error can be applied to a larger group of which these animals are a random sample only in case two-column pedigrees are used. As applied to single individuals, the formula gives the probable error of the approximate coefficient merely as a measure of the complete coefficient. It does

not, of course, give the probable error of the change in percentage of homozygosis. This is a function of the unknown number of Mendelian factors which were heterozygous in the foundation stock. The complete coefficient should give the decrease in heterozygosis very accurately for characters dependent on thousands of genes but with very little reliability in an individual case for a character dependent on only two or three genes.

RELATIONSHIP

The calculation of coefficients of relationship from random samples of pedigrees offers no additional complications of importance.

The presence of a tie between *single* random lines back of the two animals considered (X, Y) indicates a coefficient of $\frac{1 + F_a}{\sqrt{(1 + F_x)(1 + F_y)}}$.

A tie in a comparison of four-column pedigrees (four possible ties) contributes $\frac{1}{4} \left[\frac{1 + F_a}{\sqrt{(1 + F_x)(1 + F_y)}} \right]$ to the coefficient, and similarly for

larger pedigrees. In calculating the relationship of a large group to a particular animal (Y), the coefficient of inbreeding of that animal (F_y) should of course be obtained with a high degree of reliability. The coefficient (F_a) of common ancestors that frequently recur should be calculated with considerable reliability. The coefficient (F_x) for the animals of the group should be calculated once for all, preferably by the two-column method.

In this case, the denominator becomes a constant factor, and only the numerator varies for different ties. The reader will have no difficulty in extending the method to other sorts of cases, such as finding the correlation between random individuals of the breed, or within a specially choice section of the breed, or between two sections of the breed. The probable error can be calculated from the proportion of ties, and be rated up by the ratio of the coefficient to this proportion as in the case of the inbreeding coefficient.

The writers have made a number of tests of the reliability of the method. The average coefficient of inbreeding of the 64 Bates Duchesses, calculated from the complete pedigrees, was given as 40.9 per cent. The random method, using four-column pedigrees, gave 42.2 per cent with a probable error of 1.1 per cent. The observed difference (1.3 per cent) happens to be slightly greater than the probable error. It is evident that the enormously simpler approximate method gives a sufficiently accurate measure of the average inbreeding of the Duchesses as a group.

As an example of the application of the method to the inbreeding of an individual, it was found that 1,024 wholly random two-column samples of the pedigree of Favourite (252) gave a coefficient of 19.0 ± 0.52 per cent. The complete method gives 19.2 per cent.

FURTHER STUDIES ON ISOELECTRIC POINTS FOR PLANT TISSUE¹

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INTRODUCTION

Previous papers by one of the writers (5, 6)³ have indicated that plant tissue—in its absorption of water, in its absorption and retention of acid and basic dyes, and in its effect upon the reaction of buffer mixtures in which the tissue is placed—acts much like an amphoteric colloid having an isoelectric point.

The so-called isoelectric point for potato-tuber tissue in the previous experiments seemed to be near P_H 6.0, for the mycelium of *Rhizopus nigricans* near P_H 5.0, and for *Fusarium lycopersici* near P_H 5.5. These points are apparently of importance in the physiology of the organisms because a low point in the growth of *R. nigricans* (6) and of *F. lycopersici* (8) is correlated with the so-called isoelectric point when the organisms are grown on media of different hydrogen-ion concentrations. This point is also apparently correlated with the ability of *F. lycopersici* to infect tomato plants (8) grown in soils of varying hydrogen-ion concentration.

In this paper the results of further experiments on the analogy between plant tissue and an amphoteric colloid with a definite isoelectric point are presented. As has been pointed out by Michaelis (3), an ampholyte placed in weak buffer mixtures changes toward greater alkalinity the reaction of those buffer mixtures that are more acid than the isoelectric point, and changes toward greater acidity the reaction of those buffer mixtures that are more alkaline than the isoelectric point. This is because the ampholyte acts as an acid in solutions more alkaline than the isoelectric point, and as an alkali in solutions that are more acid than the isoelectric point. At the isoelectric point, the reaction of the solution in which the ampholyte is placed remains unchanged. One of the writers has to some extent used this method of determining the isoelectric point with potato-tuber tissue (5), and the mycelium of *R. nigricans* (6). The previous experiments, however, were subsidiary to those on water absorption, dye absorption, and growth. The experiments reported here were designed to follow out more elaborately with plant tissue this method of determining the isoelectric point.

PROCEDURE

The general procedure was as follows. The plant tissue was added to a small quantity of a dilute buffer mixture, and the changes in reaction were measured at intervals until equilibrium was reached. If the tissue acts like an amphoteric colloid, with a definite isoelectric

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³ Reference is made by number (italic) to "Literature cited," page 398.

point, it should change toward greater alkalinity the reaction of solutions that are more acid than the isoelectric point, and vice versa. The equilibrium point reached would depend on the relative quantities of tissue and buffer mixture, the reactive power of the tissue, and the strength of the buffer mixture. If an ampholyte were present in excess, the equilibrium point would correspond with the isoelectric point. If it were deficient, the equilibrium point would be more acid or more alkaline than the isoelectric point, depending upon which side the initial reaction lay.

The tissues used were potato-tuber tissue, root tips of Virginia soy beans, and the mycelial mats of three fungi: *Fusarium lycopersici*, *Gibberella saubinetii*, and *Fusarium oxysporum*.

The reaction of the solutions was measured electrometrically with the saturated calomel electrode, a modified Hildebrand type of bubbling hydrogen electrode, a Leeds-Northrup type K potentiometer, and type R galvanometer. The hydrogen electrode was a straight piece of platinum wire suitably coated with platinum black. A siphon bridge was used with a curved capillary for making contact with the solution in the electrode vessel, and with a wooden plug in the end in the saturated potassium chloride solution to minimize diffusion. When the wooden plug was placed in the end of the siphon in contact with the liquid in the electrode vessel, disturbances were produced which interfered with the determination of the E. M. F. The bridge was left in contact with the liquid in the electrode vessel during the entire time of the experiment. Some diffusion of the saturated potassium-chloride solution into the electrode vessel probably occurred, and, if it did, this may have been a disturbing factor in the experiments. The wooden plug and capillary, however, prevented much diffusion.

All determinations were made at temperatures which varied between 23.5° and 26.0° C. The light from an electric lamp shone on the tissue during the experiments.

EXPERIMENTAL DATA

POTATO-TUBER TISSUE

The potato-tuber tissue was prepared as described earlier (5), in disks about 1 cm. in diameter and 1 mm. thick. About 4 gms. were placed in 10 c. c. of 0.001 M. potassium dihydrogen phosphate, the reaction of which had been adjusted to the desired P_H by the addition of 0.001 M. potassium hydroxide. The electrode was surrounded by a basketwork of platinum to keep the potato disks from touching it. The electrode vessel consisted of the lower third of a 100 c. c. graduated cylinder. With the hydrogen bubbling continuously through the solution, the E.M.F. was read at intervals until an equilibrium point was reached. The tissue was then removed from the solution, and the E.M.F. determined. This was considered the final reading. In some cases a fresh lot of tissue was added to the solution in order to make certain that the equilibrium point reached was not due to the saturation of the tissue with the particular ion concerned.

The data for a typical pair of experiments performed on the same day are given in Table I. In experiment 12, a solution of P_H 6.32 became more alkaline, reaching a final equilibrium point of P_H 6.44. In experiment 13, a solution of P_H 6.69 became more acid, reaching an

equilibrium point of P_H 6.46. In experiment 12 (Table I), the P_H dropped from 6.32 to 6.26 2 minutes after the addition of the potato-tuber tissue, and at the end of 10 minutes it had reached a constant value of P_H 6.44. In experiment 13 (Table I), the addition of the potato had reduced the original P_H of 6.69 to 6.31 at the end of 3 minutes. At the end of 9 minutes, the Sørensen value of the solution had risen to 6.61 and then fallen slowly to 6.50 by the end of 60 minutes. The addition of a fresh lot of potato-tuber tissue to this solution caused a drop from P_H 6.50 to P_H 6.24 at the end of 6 minutes. At the end of 12 minutes after the fresh tissue was added the Sørensen value was 6.46, at which point it remained.

TABLE I.—Data for two typical experiments with 4.5 gms. of potato-tuber tissue placed in 10 c. c. of 0.001 M. potassium dihydrogen phosphate, the initial reaction of which was adjusted with 0.001 M. potassium hydroxide and the reaction measured until equilibrium was reached

Experiment 12		Experiment 13	
Time in minutes	P_H	Time in minutes	P_H
0	6.32	0	6.69
2	6.26	3	6.31
10	6.44	9	6.61
18	6.44	14	6.59
24	6.44	26	6.55
30	6.44	45	6.52
(*)	(*)	57	6.51
37	6.44	70	6.50
39	6.44	(b)	(b)
		76	6.24
		82	6.46
		91	6.46
		102	6.46
		(a)	(a)
		106	6.46
		109	6.46

* Tissue removed at this point.

^b Added 4.5 grams fresh potato at this point.

The drop in Sørensen units a few minutes after the addition of the potato tissue to the solution, and the subsequent recovery, was noted in all the experiments summarized in Table II, except in experiments 3, 4, and 5. In these three experiments the initial P_H was 6.14 or less, and in all others 6.32 or greater. This immediate and temporary increase in acidity on the addition of the potato-tuber tissue to the dilute buffer mixtures is probably due to carbon dioxide introduced with the tissue and swept out in a few minutes by the constantly bubbling hydrogen.

TABLE II.—Summary of experiments with potato-tuber tissue. Tissue placed in each case in 10 c. c. of 0.001 M. potassium phosphates, and reaction determined until equilibrium was reached

Experiment No.	Initial P_H	Final P_H	Time in minutes	Amount potato in grams	Experiment No.	Initial P_H	Final P_H	Time in minutes	Amount potato in grams
1.....	6.54	6.39	145	4.1	8.....	6.67	6.46	63	4.3
2.....	6.32	6.02	119	4.1	9.....	6.33	6.16	165	4.5
3.....	5.85	6.20	72	4.0	10.....	6.33	6.37	33	4.5
4.....	6.10	6.43	116	4.4	11.....	6.69	6.38	90, 31	2×4.5
5.....	6.14	6.43	99	4.3	12.....	6.32	6.44	30	4.5
6.....	6.68	6.45	54	4.3	13.....	6.99	6.46	70, 30	2×4.5
7.....	6.32	6.44	55	4.3					

The writers conclude from the two experiments presented in Table I that potato-tuber tissue in 0.001 M. potassium phosphates acts like an amphoteric colloid with an isoelectric point of P_H 6.45. On the acid side of this point the tissue acts mainly as a base, taking up the phosphate ion chiefly; and on the alkaline side it acts mainly as an acid, taking up the potassium ion.

Most of the results in 11 other experiments confirmed those of the 2 just described. In Table II are summarized 13 experiments made with potato-tuber tissue by the methods which have been given. The initial P_H of the solution, the final P_H of the solution, the length of time the experiment was run, and the weight of the potato-tuber tissue are given for each experiment. In experiments 11 and 13, the original lot of tissue was replaced by a fresh lot. Experiments done on the same day on the same sample of potato tubers are bracketed together. An examination of the data in Table II shows that in 7 cases the final equilibrium point reached was P_H 6.43 to 6.46; in 3 additional cases P_H 6.37 to 6.39. In experiment 3 the final point reached was P_H 6.2. This probably represents, however, an equilibrium point due to a deficiency of ampholyte. The solution in experiment 3 was the most acid one used and would necessitate a greater absorption to bring its reaction to the isoelectric point than would solutions such as were used in experiments 4 and 5. If a fresh lot of tissue had been added to the solution used in experiment 3 the Sørensen value would probably have been increased. In only two cases were the results contrary to the conclusion that potato-tuber tissue in 0.001 M. potassium phosphates acts like an amphoteric colloid with an isoelectric point near P_H 6.4. In experiments 2 and 9 the addition of potato-tuber tissue to solutions of P_H 6.32 and 6.33 lowered the P_H instead of raising it. Whether this is due to experimental errors, to variations in the potato tissue, or to some other cause is an unsolved problem.

Other experiments than those summarized in Table II were made with potato-tuber tissue. The results, however, were considered misleading because from 25 to 50 c. c. of solution was used in a 150 c. c. beaker and the hydrogen electrode was unprotected. This larger amount of liquid and the larger container did not permit the complete and rapid removal of the carbon dioxide by the stream of hydrogen. The potato disks crowding together on one side of the beaker prevented the ready mixing of the solution with the possible development of a local zone the reaction of which would be different from that in the vicinity of the electrode. Experiments in which potato-tuber tissue was placed in 50 c. c. of 0.001 M. potassium phosphates of initial reaction of P_H 5.73, 6.37, 6.48, and 6.97, showed final Sørensen values, after 6 to 7 hours, of P_H 6.30, 6.78, 6.70, and 6.86, respectively. Hydrogen was bubbled through the solutions part of the time during these experiments, and part of the time the potato stood quietly in the solutions. These high values are no doubt due to the presence of carbon dioxide, which kept the reaction of the tissue, or a part of it, on the acid side of the isoelectric point, and induced a continued absorption of the phosphate ion as in the experiments with soy-bean root tips described later in this paper.

The results of the experiments given in Table II agree fairly well with those performed earlier by one of the writers (5) on the effect of buffer mixtures on water absorption and dye absorption by potato-

tuber tissue. They show that potato-tuber tissue affects the reaction of 0.001 M. potassium phosphates much the same as would an amphoteric colloid with a definite isoelectric point. The so-called isoelectric point found in the present experiments is more alkaline than that found in the earlier experiments. A more dilute buffer mixture, and the potassium phosphates instead of the sodium phosphates, were used in the present experiments. It is interesting to note that Stiles and Jørgensen (10) found that potato-tuber tissue neutralized both dilute hydrochloric acid and dilute potassium hydroxide.

SOY-BEAN ROOT TIPS

Virginia soy beans were germinated between filter papers in pans, and a centimeter or two of the root tips was cut off. From 24 to 60 such root tips were placed in 10 c. c. of the 0.001 M. potassium phosphates. The electrode used was of the same type as that used in the experiments with potato, but it was not protected with the platinum basketwork, as the root tips, being smaller, were less likely to strike it.

The results of the experiments with soy-bean root tips are summarized in Table III. The results are much less uniform than with potato. Solutions of initial P_H of 6.68, 6.69, and 6.78 were made more acid. Solutions of initial P_H of 5.82 and 6.20 were made more alkaline. In three cases, solutions of P_H 6.32 or 6.33 became more alkaline, and in four cases more acid. The lowest Sørensen unit reached from the alkaline side was P_H 5.89, and the highest from the acid side was P_H 6.44. Considering experiments performed on a single day, such as 1 and 2, 5 and 6, and 9 and 10, the root tips affected the solutions as an ampholyte would be expected to affect them. The majority of the experiments indicate that the isoelectric point for soy-bean root tips lies in the vicinity of P_H 6.20 to 6.44.

TABLE III.—Summary of experiments with Virginia soy-bean root tips. Tissue in each case placed in 10 c. c. of 0.001 M. potassium phosphates and reaction determined until equilibrium was reached

Experiment No.	Time in days from beginning of germination	Initial P_H	Final P_H	Time in minutes	Weight of tissue in grams	Experiment No.	Time in days from beginning of germination	Initial P_H	Final P_H	Time in minutes	Weight of tissue in grams
1.....	7	6.20	6.43	45, 25	2×0.7	6.....	7	6.32	6.19	100	1.5
2.....	7	6.78	6.42	34, 54, 38, 108	4×0.7	7.....	5	6.32	6.38	116	1.5
3.....	4	6.32	6.34	127	0.8	8.....	5	5.82	6.18	112	1.5
4.....	5	6.68	6.19	180, 49	2×0.9	9.....	6	6.33	6.44	93	1.3
5.....	7	5.82	6.13	39, 25, 20, 23	4×1.5	10.....	6	6.69	6.49	150, 18	2×1.3
						11.....	7	6.33	5.96	191	1.3
						12.....	8	6.32	5.89	164	2.3

The writers do not know why the results of successive experiments with the soy-bean root tips are not duplicates. One factor may be the age of the root tips; another may be the fact that meristematic tissue was being dealt with as well as partially or completely differentiated tissue, the proportions varying in the different lots of root tips. Some extremely interesting observations were made on the effect of the acidity due to carbon dioxide on the changes in reaction pro-

duced by the root tips. In the experiments in which hydrogen was bubbled continuously through the liquid in which the soy-bean root tips were placed, the final reaction reached from the acid side (Table III) was never more than P_H 6.44. If the root tips were allowed to stand undisturbed in the liquid, the P_H measured colorimetrically quickly approached 5.9, at which point it remained constant. If the reaction of the solution were then determined electrometrically, it was found to be near P_H 6.7. This is illustrated by the following experiment.

Sixty root tips of Virginia soy beans, totaling about 3.2 gms., were placed in 25 c. c. of 0.001 M. potassium phosphates in a 150 c. c. beaker and allowed to stand quietly. The reactions of the solutions were read colorimetrically. Within 10 minutes a solution of original P_H 5.6. had changed to P_H 5.9, and it remained unchanged in reaction for 90 minutes, during which time determinations of the hydrogen-ion concentration were made colorimetrically at intervals. After 60 minutes a solution of original P_H 6.1 had changed to P_H 5.9. After 90 minutes the reactions of the two solutions, which were P_H 5.9 colorimetrically, were determined electrometrically. The values were P_H 6.72 and 6.85, respectively. The solutions were removed from the electrode vessels and the reaction measured colorimetrically. The results were P_H 6.7 and 6.9, respectively.

These results were evidently due to the carbon dioxide produced by the respiration of the root tips. Standing quietly in the solutions in the beakers, carbon dioxide given off by the root tips accumulated in the solutions and kept the reaction near P_H 5.9. This point, as indicated by the majority of the data in Table III, is acid to what the writers have called the isoelectric point. The absorption of the phosphate ion therefore continued. When the electrometric determination was made, the carbon dioxide in the solution was swept out by the hydrogen, and the reaction in the absence of the carbon dioxide was found to be P_H 6.7 to 6.9. If the carbon dioxide had not been present, the reaction would probably have slowly changed in both solutions to approximately P_H 6.4, at which point the change would have ceased. Evidently acidity produced by carbonic acid affects the relative amounts of cations and anions absorbed, just as does the acidity produced by phosphoric acid.

MYCELIAL MATS OF GIBBERELLA SAUBINETII

This fungus, as well as the two others described later in this paper, was grown on the mineral nutrient solution used by one of the writers (8) with a total concentration of 0.066 M. monobasic and dibasic potassium phosphate and an initial P_H of 5.0. The mycelial mats were removed from the nutrient solutions and thoroughly washed with 0.2 M. cane-sugar solution or redistilled water. The excess liquid was pressed out of the mats, which were placed in the dilute buffer mixtures, where readings were taken, with the hydrogen bubbling continuously until equilibrium was reached.

This strain of *Gibberella saubinetii* was one isolated by B. B. Branstetter from corn. The results obtained with this organism are summarized in Table IV, and Figure 1 shows curves with the P_H of the solutions on the ordinate and the time on the abscissa.

TABLE IV.—Summary of results with mycelium of *Gibberella saubinetii*. Tissue in each case placed in solution indicated and reaction measured until equilibrium was reached

Experi- ment No.	Weight of myce- lium	Age of myce- lium	Kind of buffer solution used	Quantity of buffer used	Initial P _H	Final P _H	Time required to reach equilib- rium
	Grams	Days		C. c.			Minutes
1	5.67	15	0.002 M. Na phosphates	50	4.03	6.14	60
2	4.40	15	0.001 M. Na phosphates	50	4.37	6.05	65
3	4.49	16	0.001 M. KH phthalate+H ₃ PO ₄	25	3.15	6.23	21
4	1.51	16	0.001 M. Na phosphates	25	5.88	6.25	19
5	1.88	11	0.001 M. Na phosphates	25	6.68	6.21	77
6	4.25	14	0.001 M. Na phosphates	50	7.88	6.34	126
7	4.65	16	0.001 M. Na phosphates	50	7.42	6.36	103

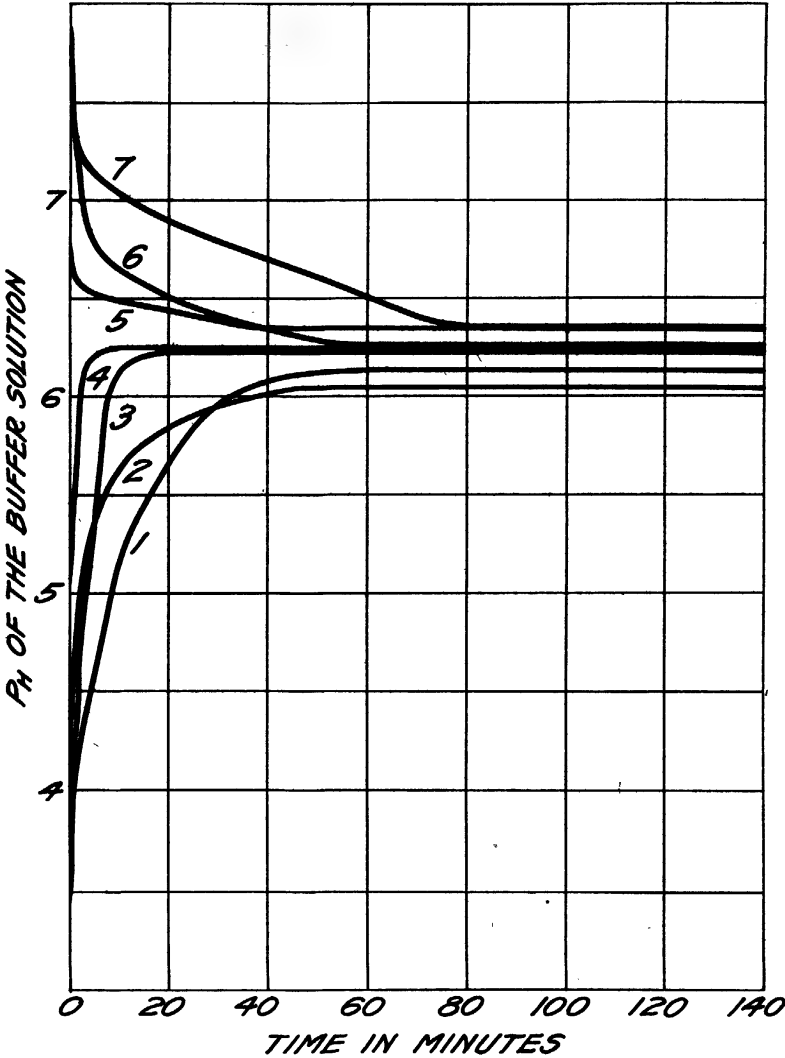


FIG. 1.—Curves showing the effect of the mycelium of *Gibberella saubinetii* upon the reaction of dilute buffer mixtures P_H of the buffer mixture is plotted against the time. The numbers on the curves refer to the experiments summarized in Table IV

The effect of the mycelium of *G. saubinetii* upon the dilute buffer mixtures was similar to that produced by an ampholyte with an isoelectric point near P_H 6.2. Solutions of initial P_H 3.15, 4.03, 4.37,

and 5.88, became more alkaline because of the mycelium; solutions of initial P_H 7.88, 7.42, and 6.68 became more acid. The lowest final P_H reached from the alkaline side was 6.21, and the highest P_H reached from the acid side was 6.25. The curves in Figure 1 show very clearly how the mycelium of this fungus brought the reaction of the buffer mixtures from both the acid and alkaline side toward P_H 6.2.

For the most part, the action of the fungus mycelium in changing the reaction of a solution was greater and more rapid than that of the potato-tuber tissue or soy-bean root tips. This was also true of the other two fungi used. It may be due to the more ready access of the liquid to the hyphae of the fungus rather than an actual difference in the action of the cell contents.

The greater rapidity with which equilibrium was reached in the phthalate buffer mixture is of interest. Comparing experiments 2 and 3, it is evident that approximately the same amount of mycelium required 65 minutes to shift the reaction of 0.001 M. sodium phosphates from P_H 4.37 to 6.05 and only 21 minutes to make a greater change in a 0.001 M. phthalate buffer mixture. This may be due to the cell contents of the fungus being already partially saturated with the phosphate ion, since it was grown in a nutrient solution containing phosphates, while its reactivity for the phthalate ion would be entirely unsatisfied.

The isoelectric point found in these experiments for *Gibberella saubinetii* is somewhat more alkaline than that which might be deduced from the growth experiments and infection experiments carried on with this organism by Hopkins (2). He found a minimum in the growth curve at P_H 5.5 to 6.0 when *G. saubinetii* was grown in solutions of various acidities and a minimum infection of wheat seedlings in soils with artificially adjusted reactions at P_H 5.5. These minima have been interpreted by one of the writers (5) to be due to the decreased water absorption which occurs at the isoelectric point. The solutions, however, used by Hopkins were complete nutrient solutions and much stronger in buffer salts than those the writers have used. Moreover, the strain of the fungus was not the same. These facts may account for the difference.

TABLE V.—Summary of results with mycelium of *Fusarium lycopersici*. Tissue in each case placed in solution indicated, and reaction measured until equilibrium was reached

Experiment No.	Weight of mycelium	Age of mycelium	Kind of buffer solution used	Quantity of buffer solution used	Initial P_H	Final P_H	Time required to reach equilibrium
	Grams	Days		C. c.			Minutes
1	0.71	8	0.01 M. KH phthalate	10	3.94	4.88	30
2	2.14	14	0.002 M. Na phosphates	50	4.03	5.50	70
3	1.50	8	0.001 M. KH phthalate	10	3.95	5.47	11
4	1.50	8	0.001 M. KH phthalate	10	3.95	5.41	16
5	5.08	7	0.001 M. Na phosphates	50	4.55	5.41	56
6	4.04	9	0.001 M. Na phosphates	50	5.43	5.48	54
7	0.635	5	0.001 M. Na phosphates	10	6.66	5.46	39
8	1.63	4	0.001 M. Na phosphates	10	7.20	5.52	34
9	¹ 0.94	16	0.001 M. Na phosphates	25	6.02	6.16	20
10	4.92	7	0.001 M. Na phosphates	50	7.20	6.00	60

¹ Autolyzed.

MYCELIAL MATS OF *FUSARIUM LYCOPERSICI*

The results obtained with the mycelial mats of *Fusarium lycopersici* are summarized in Table V. In Figure 2, curves of the results are given with the P_H of the solutions on the ordinate and time on the abscissa.

The mycelium of *F. lycopersici* affects the buffer mixtures used like an ampholyte with an isoelectric point at P_H 5.5. Solutions of initial reaction P_H 3.94, 3.95, 4.03, 4.55, and 5.43 became more alkaline because of the presence of this fungus, while solutions of initial P_H 7.20 and 6.66 became more acid. The results of experiment 9 can be overlooked in drawing conclusions, because the 16-

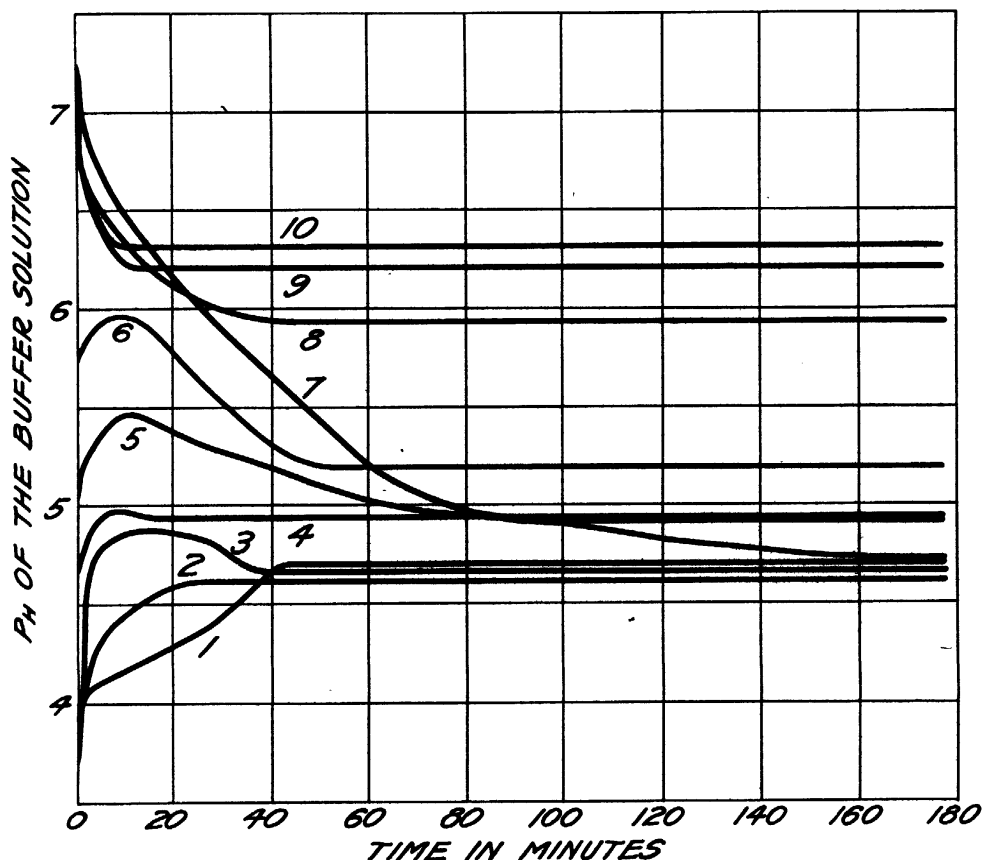


FIG. 2.—Curves showing the effect of the mycelium of *Fusarium lycopersici* upon the reaction of dilute buffer mixtures. The P_H of the buffer mixture is plotted against time. The numbers on the curves refer to the experiments summarized in Table V

day old mycelium used in this experiment was badly autolyzed. Disregarding this experiment, the lowest P_H reached from the alkaline side was P_H 5.52, and the highest from the acid side was P_H 5.50.

Again the much greater rapidity of action of the mycelium in changing the reaction of phthalate buffer mixtures, as compared to the phosphate solutions occurred. In experiment 4 it required 16 minutes for 1.50 gms. of the mycelium to change an 0.01 M. phthalate solution of P_H 3.95 to a P_H of 5.41; while, in experiment 2, 2.14 gms. of mycelium required 70 minutes to make a smaller change in the reaction of a 0.002 M. phosphate buffer mixture. This difference in speed of action is brought out clearly by the much steeper slope of the curve for experiment 4 as contrasted with that for experiment 2, as shown in Figure 2.

The changes in reaction in experiment 6 did not follow the regular order of the balance. Instead of proceeding uniformly toward an equilibrium point, the mycelium changed the reaction to above P_H 5.50, then to a point below that value, followed by a second oscillation toward greater alkalinity before the reaction settled to an equilibrium point at P_H 5.48. This is shown by the curve for experiment 6 in Figure 2. The initial reaction of the solution in experiment 6 was more nearly the isoelectric point than any other used. This fact may be connected with the peculiar action of the mycelium in this experiment.

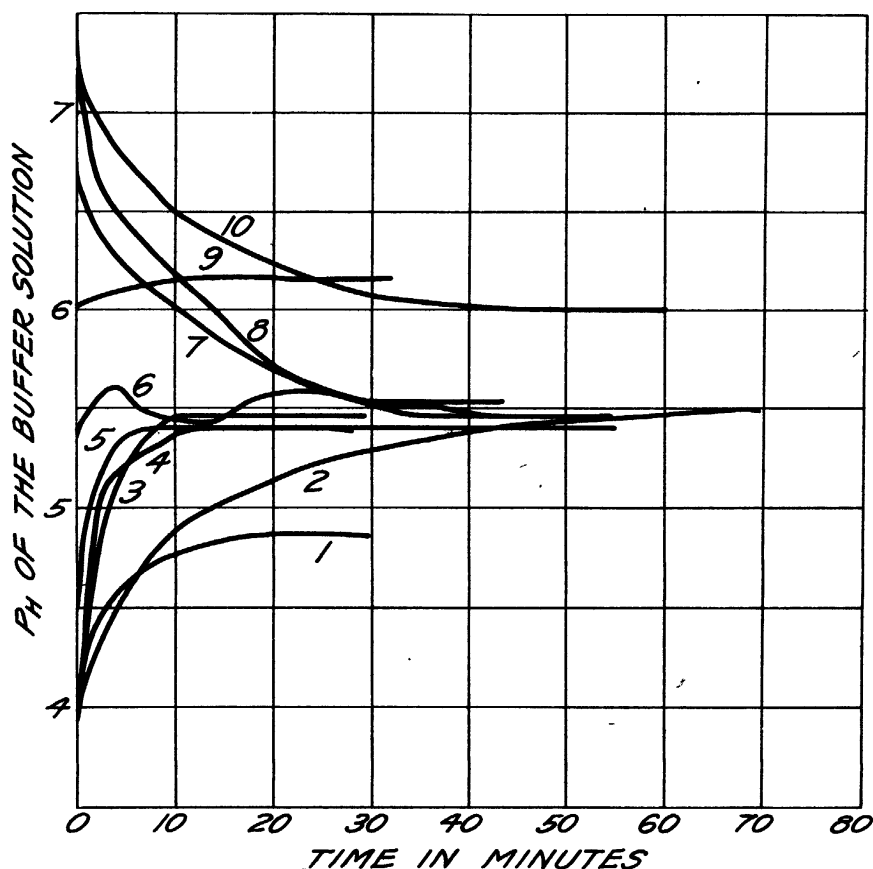


FIG. 3.—Curves showing the effect of the mycelium of *Fusarium oxysporum* upon the reaction of dilute buffer mixtures. The P_H of the buffer mixture is plotted against time. The numbers on the curves refer to the experiments summarized in Table VI

The isoelectric point found for the mycelium of *Fusarium lycopersici* in these experiments agrees very well with that found earlier in studies on the influence of hydrogen-ion concentration on dye absorption (6), and on the growth of the same strain of this organism (8).

MYCELIAL MATS OF FUSARIUM OXYSPORUM

The results of experiments with *Fusarium oxysporum* are summarized in Table VI and Figure 3.

The mycelium of *F. oxysporum* acts much like an ampholyte with an isoelectric point near P_H 4.9. Solutions of initial P_H 3.80, 4.02, 4.04, and 4.73 became more alkaline because of the presence of the mycelium; while solutions of initial P_H 7.36, 7.10, 6.94, 5.70, and 5.06 became more acid. The highest final P_H reached from the acid side was 4.94, and the lowest from the alkaline side was 4.71.

In the curves of Figure 3, a peculiarity of the results of some of the experiments (3, 4, 5, and 6) with this fungus is evident. In all four of these experiments the initial effect of the mycelium was to produce an alkalinity greater than the final equilibrium point, forming humps on the curves.

TABLE VI.—Summary of results with mycelium of *Fusarium oxysporum*. Tissue in each case placed in the solution indicated, and reaction measured until equilibrium was reached

Experiment No.	Weight of mycelium	Age of mycelium	Kind of buffer solution used	Quantity of buffer solution used	Initial P_H	Final P_H	Time required to reach equilibrium
	Grams	Days		C. c.			Minutes
1.....	1.03	17	0.01 M. KH phthalate.....	10	4.02	4.70	40
2.....	1.13	17	0.01 M. KH phthalate.....	10	4.04	4.63	38
3.....	3.17	9	0.001 M. Na phosphates.....	50	3.80	4.64	52
4.....	0.50	15	0.0005 M. Na phosphates.....	25	4.73	4.94	19
5.....	0.90	17	0.001 M. KH phthalate, KOH.....	10	5.06	4.71	166
6.....	1.46	9	0.001 M. Na phosphates.....	10	5.70	5.195	54
7.....	1.39	9	0.001 M. Na phosphates.....	10	7.36	4.92	101
8.....	2.282	11	0.001 M. Na phosphates.....	50	6.94	5.94	58
9.....	1.50	11	0.001 M. Na phosphates.....	10	7.10	6.21	15
10.....	0.47	11	0.001 M. Na phosphates.....	5	7.10	6.31	12

DISCUSSION

The experiments reported here show that under the conditions of these experiments, plant tissue acts much like an ampholyte with a definite isoelectric point. For potato-tuber tissue this point is near P_H 6.4, for the mycelium of *Gibberella saubinetii* near P_H 6.2, for that of *Fusarium lycopersici* near P_H 5.5, and for *Fusarium oxysporum* near P_H 4.9. For the root tips of soy beans it is in the neighborhood of P_H 6.2 to 6.4, but further work is necessary to define it more exactly, if possible.

The decided difference in the so-called isoelectric points for these three closely related fungi suggests the possibility of using this method or one similar to it as an aid in identification.

The experiments also emphasize the importance of the acidity produced by carbonic acid. If the writers' interpretation of the experiments is correct, the accumulation of this acid can profoundly affect the absorption of ions by the cell, and probably, also, other physiological processes which depend upon reaction with the isoelectric point as the critical one. Most of the isoelectric points reported here are in the zone in which carbonic acid can affect the reaction. This suggests the importance of considering the carbon-dioxide content of the cell contents when the hydrogen-ion concentration of plant tissue is determined. Apparently most determinations of the hydrogen-ion concentration of plant tissues are now made on the extracted sap with no attention paid to the carbon-dioxide content of the juice. Yet the acidity due to carbon dioxide may be as important in plant juices as in the blood of animals. Its accumulation in the tissues of higher plants at night when the stomates are closed might throw the reaction across the isoelectric point or move it further away with possible profound physiological effects.

The methods used in the experiments reported here need further refinement; and a study of the effect of various conditions, such as

other buffer mixtures, light, age, death, temperature, and dissolved materials, should be made. The main thesis, however, that plant tissue acts much like an amphoteric colloid with definite isoelectric points appears clear, substantiated as it is by earlier work by the writers. Stearn and Stearn (9) have come to somewhat similar conclusions in their work on the staining of bacteria.

At the same time several facts also show that plant tissue does not act completely like a simple protein such as gelatin. The overlapping of the final equilibrium points noted in the experiments described here with all the tissues, except that of *F. lycopersici*, would not be expected with gelatin. It must be remembered, however, that protein and other materials of plant tissue are inclosed in a cellulose wall, and that the reaction in the cell may not be that of the solution in which it is immersed. The shifting of the so-called isoelectric point caused by ions other than the hydrogen and hydroxyl ions, noted in the earlier experiments on water absorption by potato-tuber tissue (5) in dilute buffer mixtures, would not occur if we were dealing with the isoelectric point of gelatin. The overlapping of the zones of absorption and retention of basic and acid dyes in plant tissue placed in solutions of different hydrogen-ion concentrations, noted by Rohde (7) and by one of the writers for living tissue, and by Naylor⁴ for dead tissue does not occur with pure gelatin. The absorption of both anions and cations on both sides of the isoelectric point must occur, else organisms could not exist as they do over a comparatively wide range of hydrogen-ion concentration. This is not what would be expected from a substance like gelatin.

What happens in the simple buffer mixtures used in the experiments reported in this paper is probably much similar to what Rohde found to be true for the influence of hydrogen-ion concentration on the absorption of dyes, and pictured so clearly in the curves he presents. On the acid side of what we have called the isoelectric point, both anions and cations are taken up, the former, however, more rapidly and in greater amount. The further we move toward greater acidity away from the isoelectric point, the more anions and the fewer cations are absorbed. On the alkaline side of the isoelectric point the situation is reversed.

In plant tissue we are probably dealing with several amphoteric colloids which have different isoelectric points. Owing to their amphoteric properties, these substances may interact with one another and form one or more amphoteric compounds which may be necessary to life. That the constituents of the cell do not all act alike, at least when dead, has been shown by Naylor. With sections of root tips he has found that the cytoplasm, resting nucleus, chromosomes, and nucleolus possess different isoelectric points.

We may also be considering not the isoelectric point but the point of maximum undissociated ampholyte molecules, the ρ maximum of Michaelis (3). The isoelectric point, as shown by Michaelis, is not affected by salt formation, but the ρ maximum is affected by salt formation. The hydrogen-ion concentration for it may coincide with the isoelectric point, or may lie on either side of it, depending upon the dissociation constants of the salts formed. This possibility is sug-

⁴NAYLOR, E. E. THE EFFECT OF THE HYDROGEN-ION CONCENTRATION UPON THE STAINING OF PLANT TISSUE BY BASIC AND ACID DYES. 1924. [Unpublished thesis, Univ. Mo.]

gested by the differences found in the position of the isoelectric point for potato-tuber tissue, as defined by the water absorption in different buffer mixtures.

Whatever may be the ultimate complete and correct explanations of these writers' data, the conception of the plant cell as an amphoteric colloid is one which will prove increasingly valuable. Pearsall and Priestley (4), and Weber (11), have suggested theories for the existence or formation of meristem based upon such a conception. Arrhenius (1), in studying the ammonia, nitrate and total nitrogen content of soils of varying natural hydrogen-ion concentration, found that while the total nitrogen was rather constant in amount, the ammonia content was at its maximum in the acid soils and the nitrate in the alkaline soils. When plotted against the P_H of the soil, the two curves for nitrates and ammonia content intersect near P_H 6.5. This is what we should expect, if plants absorbed ions as they are taken up by an ampholyte with an isoelectric point near P_H 6.5. Nitrate, as an anion, would be absorbed to the greatest extent from soils more acid than P_H 6.5, and ammonia, a cation from soils more alkaline than P_H 6.5. The maximum amount of nitrate would be left in alkaline soils and the maximum amount of ammonia in the acid soils. The isoelectric points given in this paper for potato-tuber tissue and soy-bean root tips are close to this value.

Speculation, however, seems to the writers as somewhat futile, on the basis of the experimental facts they now have. More complete and more careful data from a variety of viewpoints are needed. The writers are convinced, however, that most of the difficulties in problems of salt absorption, water absorption, "permeability," toxicity, and antagonism can be solved, and the mass of data concerning them unified by considering plant tissue as an amphoteric colloid which reacts with ions according to chemical laws, rather than as a system of osmotic chambers with membranes of changeable permeability.

SUMMARY

When potato-tuber tissue was placed in small quantities of 0.001 M. buffer mixtures of the potassium phosphates, and the reaction was determined electrometrically with the hydrogen bubbling continuously, solutions of P_H 6.5 or greater became more acid, and solutions of P_H 6.14 or less became more alkaline. Solutions of P_H 6.32 or 6.33 became more alkaline in some cases and more acid in others.

In the majority of cases the equilibrium point reached with potato-tuber tissue under the conditions just referred to was P_H 6.37 to 6.46. The lowest equilibrium point reached from the alkaline side was P_H 6.02, and the highest from the acid side was P_H 6.44.

When allowed to stand quietly for part of the time in larger quantities of the buffer mixtures, the final reaction reached, as determined electrometrically, was P_H 6.3 to 6.86.

When soy-bean root tips were placed in small quantities of 0.001 M. buffer mixtures of the potassium phosphates, and the reaction was determined electrometrically with the hydrogen bubbling continuously, solutions of P_H 6.68 or greater were made more acid and solutions of P_H 6.2 or less were made more alkaline. Solutions of P_H 6.32 or 6.33 became more acid in some cases and more alkaline in others.

In the majority of cases the equilibrium point reached with soy-bean root tips under the foregoing conditions was P_H 6.18 to 6.49. The lowest equilibrium point reached from the alkaline side was P_H 5.89, and the highest from the acid side was P_H 6.44.

When allowed to stand quietly in larger quantities of the buffer mixtures, the equilibrium point reached, as determined colorimetrically, was P_H 5.9; as determined electrometrically, P_H 6.72 to 6.85.

When the mycelium of *Gibberella saubinetii* was placed in small quantities of dilute buffer mixtures, and the reaction determined electrometrically, solutions of P_H 5.88 or less became more alkaline, and solutions of P_H 6.88 or greater became more acid. The lowest equilibrium reached from the alkaline side was P_H 6.21, and the highest from the acid side was P_H 6.25.

When the mycelium of *Fusarium lycopersici* was treated in the same way, solutions of P_H 5.43 or less became more alkaline, and solutions of P_H 6.66 or greater became more acid. The lowest equilibrium point reached from the alkaline side was P_H 5.52, and the highest from the acid side, with one exception, was P_H 5.50.

When the mycelium of *Fusarium oxysporum* was treated in the same way, solutions of P_H 4.73 or less became more alkaline, and solutions of P_H 5.06 or greater became more acid. The lowest equilibrium point reached from the alkaline side was P_H 4.71, and the highest from the acid side was P_H 4.94.

It is concluded that, under the particular conditions of these experiments, these plant tissues act much like amphoteric colloids, with isoelectric points as follows: For potato-tuber tissue at P_H 6.4; for soy-bean root tips (variety Virginia) at 6.2 to 6.44; for the mycelium of *Gibberella saubinetii* at 6.2; for the mycelium of *Fusarium lycopersici* at 5.5; and for the mycelium of *Fusarium oxysporum* at 4.9.

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No. 5

THE MINIMUM TEMPERATURE FOR GROWTH OF THE
DATE PALM AND THE ABSENCE OF A RESTING
PERIOD¹

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INTRODUCTION

The culture of the date palm is a relatively new industry in the United States, though scattering trees grown for ornamental purposes or for domestic fruit supply have been known for many years in Southern California and Arizona and in the Gulf Coast regions. The first established date gardens in the United States where systematic observations could be made on the relation of the date palm to climatic environment were planted during the early years of the present century. The date-growing regions of the Old World are generally so remote from the centers of scientific research that definite published observations on the temperatures have been meager. The empirical knowledge of the natives as to the success or failure of their date varieties under different climatic conditions is often very accurate, but as yet little of this knowledge has been coined into a general circulating medium. In our own country the mistake is often made of assuming that where the date palm as a tree is successfully grown its commercial culture for fruit must be equally successful.

Twenty years of observation of the date palm in relation to temperature have established clearly the fact that *Phoenix dactylifera* as a species represented by the cultivated varieties has very definite temperature requirements which must be provided, often within very narrow limitations, if cultural success with a particular variety is to be secured. The zero point, or the temperature below which the growth of date palms ceases with prolonged exposure, seems to be the foundation upon which all study of its other temperature relations should be based. The relative temperature efficiency of two localities in which the date palm is able to grow will be measured most accurately, not by the summation of heat units above zero F. or zero C. but above the zero point of the date palm's activity, which has heretofore been but imperfectly known. For this reason the writer's studies of the zero point of the date palm, both under field and laboratory conditions, are offered here in advance of the studies of the more general relations of this species to climatic conditions.

¹ Received for publication Sept. 26, 1924; issued October, 1925.

THE ZERO POINT OF VEGETATIVE GROWTH

The accurate determination of the zero point, or specific temperature below which all growth of the date palm would cease, is a laboratory problem involving the possibility of the nicest temperature control and the elimination of the effect of sudden temperature variations. With the most favorable conditions for experimenting provided, it would probably be found that the zero point is not constant for the species but varies with the varieties and with the individual plants, so that if a zero point for the species were assumed from results of the study of one variety, it might be but a matter of time until it would be demonstrated that another variety begins its growth at a slightly lower temperature.

For the purpose of this paper, cessation in pushing up of the inner leaves from the bud of a date palm at the normal period of the day will be assumed to indicate a dormant condition of that tree.

De Candolle evidently expected the date tree to enter a resting period somewhat independently of temperature conditions, for he writes regarding it as follows: ²

Vegetation probably ceases to be active during the winter, but on a tree which retains its leaves this period is necessarily not well defined. . . . Its vegetation ought to be greatly retarded after fruiting. It is the ordinary physiological law, furthered by the lowering of the temperature. For how long does this resting last? At what average heat will the activity of the tree begin again in the spring? This is what is necessary to know, but positive observations along this line are lacking.

The growth records at Indio, Calif., and at Tempe, Ariz., however, show that growth ceases only (1) under destructive minimum temperatures of about 20° F. or lower, and (2) when zero point maximum temperatures are reached. With accurately marked active leaves under daily observation at the Indio date garden, it was determined that slow growth was made when minimum temperatures dropped below the freezing point, even down to 22° or 21°, provided the day's maximum temperatures were well above 50°. This proved that such low temperatures, if not prolonged, did not in themselves wholly check growth. The short duration usual to such minima prevents penetration to the growth center of the tree. If, now, with minimum temperatures above the freezing point, low maximum temperatures occur, followed by cessation of growth, this result may be logically attributed to the low maxima and a zero point indicated.

ZERO POINT RECORDS AT INDIO, CALIF.

In the autumn of 1916 records of daily leaf growth were begun on four date palms in the United States date garden at Indio, Calif., the growth data in the accompanying tables being the average daily pushing up from the bud center of five active young leaves. These growth records could be coordinated with standard thermometer and thermograph records taken in a standard weather bureau shelter a short distance from the trees. In these records the writer was fortunate in obtaining, in December, 1916, and January, 1917, records of temperatures associated with a sharp decline and finally

² CANDOLLE, ALPH. DE. *GÉOGRAPHIE BOTANIQUE RAISONNÉE*. v. 1, p. 371. Paris. 1855.

the complete cessation of growth in the date palms under observation, which afford the closest approximation to the specific zero point that has yet been secured under field conditions.

After the mild weather and free growth of November and early December, the temperatures from December 7 were decidedly lower (fig. 1, Table I). For 12 days, from December 8 to 19, inclusive, while the mean maximum was still 68.8° F., there were 11 minimum records below 32° and the mean of the minima was 27.8°. There were 4 mornings with a minimum of 25°, and the lowest point reached was 21° on December 10. The retarding influence of successive minima almost continuously below the freezing point was very evident in the early part of this period.

TABLE I.—Temperature and daily growth records of varieties of date palm, Indio, Calif., December, 1916

[Critical low temperatures and approximation to zero of growth are indicated by boldface type]

Date	Temperature			Mean daily growth of varieties			
	Maxi- mum	Mini- mum	Mean	Asherasi	Deglet Noor	Thoory	Zaheedy
	° F.	° F.	° F.	Mm.	Mm.	Mm.	Mm.
Dec. 1	73	44	58.5	12.7	11.70	9.20	5.20
2	72	41	56.5	11.9	13.50	9.70	4.60
3	76	55	65.5	7.6	11.70	9.70	4.50
4	75	52	63.5	13.0	14.00	10.00	6.70
5	79	47	63.0	12.5	14.50	11.10	8.00
6	73	48	60.5	12.0	15.30	10.90	8.00
7	61	35	48.0	7.0	11.40	10.40	7.40
8	62	26	44.0	10.7	12.30	10.50	5.70
9	65	25	45.0	7.0	6.90	4.80	2.70
10	68	21	44.5	3.6	2.60	4.10	1.50
11	65	25	45.0	2.7	1.90	2.40	.50
12	67	25	46.0	5.4	1.60	2.50	1.00
13	74	30	52.0	5.0	.80	2.10	.25
14	79	35	57.0	3.2	1.20	1.60	.75
15	73	28	50.5	3.4	2.20	1.80	.90
16	73	25	49.0	3.4	2.10	1.10	1.30
17	69	28	48.5	2.4	2.80	2.40	1.06
18	72	31	51.5	5.4	1.60	1.60	1.06
19	67	27	47.0	5.6	3.00	1.70	1.00
20	65	40	52.5	3.2	.65	1.16	.80
21	71	34	52.5	5.9	1.90	1.75	.40
22	68	37	52.5	4.5	2.60	1.80	1.10
23	65	36	50.5	3.6	2.10	1.10	1.20
24	68	40	54.0	2.7	2.50	1.75	.70
25	55	38	46.5	2.7	2.50	1.75	.70
26	48	30	39.0	2.7	2.50	1.75	.70
27	53	22	42.5	.4	.43	.30	.12
28	52	28	40.0	1.2	1.30	1.60	.12
29	58	38	48.0	.8	.21	.16	.80
30	67	38	52.5	.0	.65	.07	.00
31	59	29	44.0	(a)	(a)	(a)	(a)
	b 66.8	b 34.4	b 50.6	-----	-----	-----	-----

• No record obtained. b Mean for the month.

The decline was most marked after the minimum temperature of 21° F. on December 10, and by December 13 the Deglet Noor and Zaheedy trees showed but a fraction of a millimeter in growth, proving that for the conditions in which they were at that period 21° was very nearly a growth-inhibiting minimum.

With the rise of the means from December 13 to 24 a little recovery was made in spite of several frosty nights, so that the depression in growth from December 26 and 27 practically to zero of growth on the

30th must be credited to the lower maxima. As the minima of this period were from 28° to 39° F., a little higher than those of the recovery period from December 13 to 18, the way is fully cleared for a zero point determination. The sharp check in growth was recorded on the morning of the 27th following the maximum of 48° at 2 p. m. on the 26th. A little recovery was made on the 28th, but the record of the night following shows a check in growth to a fraction of a millimeter on all trees, and all but the Deglet Noor tree show practically zero point of growth on the 30th. It is evident that the conditions of the observations do not enable one to decide positively whether it required all of the 48° to inhibit growth, or whether 50° or 51°

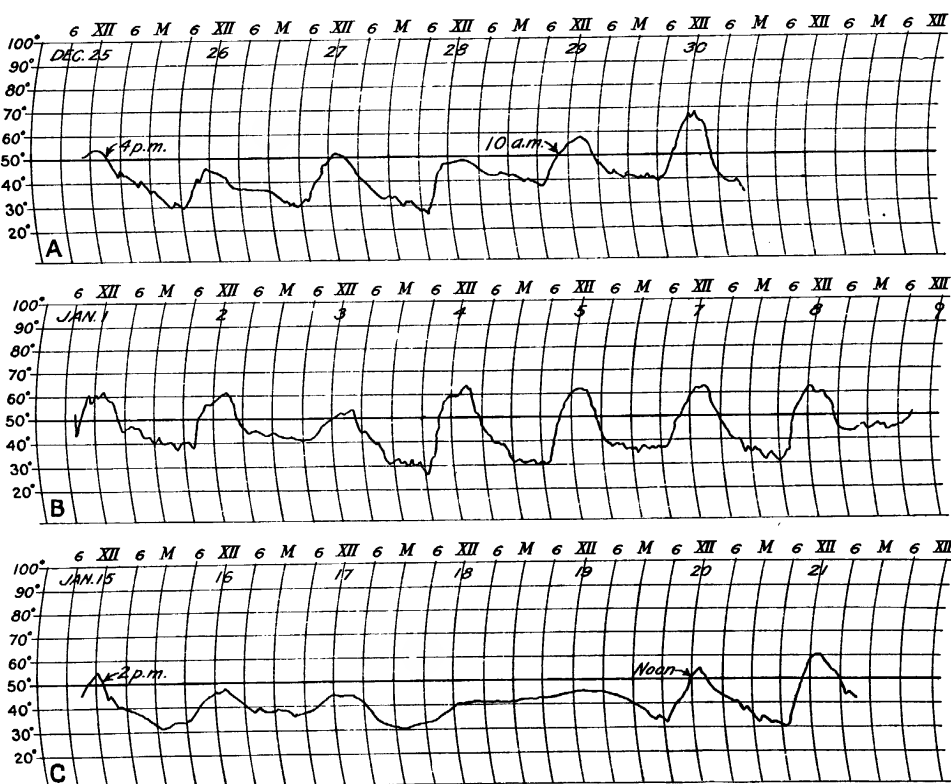


FIG. 2.—Transcripts and thermograph records at the United States date garden, Indio, Calif., covering the critical periods in zero point determination of Tables I and II and Figure 1. A, the hours when the temperature was below 50° F., December 25 to 30, inclusive; B, a similar record for January 1 to 9, and C, for January 15 to 21

would have accomplished this result. This period must be given its weight with the other records.

The period of exposure of the plants to a temperature of 50° F. or below was longer than is apparent from the tables. Reference should here be made to the transcript from the thermograph sheet for December 25 to 30 (fig. 2, A) which, while varying a little from the records of the standard maximum and minimum thermometers, affords on an occasion like this a most illuminating exhibit of the temperatures to which the plants were actually exposed. Assuming the minimum temperature for growth to be 50° , the graph shows for how long a period the temperature was below the 50° line and how little value the very short period of 2 or 3 degrees above 50° in the middle of the day could have had.

INDIO THERMOGRAPH RECORD, DECEMBER 25-29, 1916

The thermographic record at the Indio date garden for the week ending January 1 shows that the temperature fell to 50° F. at about 4 p. m. December 25, and remained below 50° until 10 a. m. on the 29th, except for brief periods on the 27th and 28th, giving a total as follows:

Summation, 50° F. or below:	Hours
December 25.....	8
December 26.....	24
December 27.....	21
December 28.....	23
December 29.....	10
Total.....	86

The January records shown in Table II throw still stronger light on this question. After the entire cessation of growth December 31, all the varieties except the Zaheedy made a slight recovery at once when the maximum temperature rose above 60° F. and there were no frosts. There was a minimum of 28° on January 4 and a record of heavy frost and a minimum of 32° on January 5. With daily maxima of 64° it is difficult to explain a drop to zero in growth of all but the Asherasi on the 7th without the aid of the thermograph record, but an analysis of the thermograph trace for seven days makes the action clear (fig. 2, B). The temperature fell below 50° at 5.30 p. m. December 31, and until 6 a. m. January 7, when the zero of growth was again recorded, there were 159½ hours, of which for 115½ hours the temperature was below 50° and 44 hours (or only 27 per cent of the time) was above 50°. Here was a case where without severe minimum temperatures the long hours below 50° prevailed over the short hours of the day temperatures above 50°. Here the mean temperatures of from 46° to 51° became zero point temperatures, though from January 15 to 21 a mean of 44° was needed to accomplish the same result.

Increasingly warm days through the 14th, with maxima ranging from 69° to 80° F. and minima well above the frost line, except 29° on the morning of the 14th, brought a return of activity so that daily growths of from 1 mm. to 4 or 5 and even exceptional cases of 6 to 9 mm. were recorded.

The 12 days' record from January 14 to 25, inclusive, is so significant as to deserve a close study in the table.

The four days January 16 to 19, inclusive, with minimum temperatures of 33° to 44° but with their maximum of only 46° to 50° F., a second time gives us the clue to the zero point, for after a lag of two days growth entirely ceased on the Zaheedy tree (apparently having the highest zero point of the four) on the 18th. Deglet Noor and Thoory both showed slight growth gains on the 18th, 19th, and 20th, but came to a full stop on the 21st. The zero point in temperature had evidently again been reached, and the four days at 50° and below are as near that point as can be determined under field conditions.

TABLE II.—Leaf growth record of date palms at Indio, Calif., January, 1917
[Critical low temperatures and approximation to zero of growth are indicated by boldface type]

Date	Temperature			Average leaf growth per day			
	Maxi- mum	Mini- mum	Mean	Asherasi	Deglet Noor	Thoory	Zaheedy
	° F.	° F.	° F.	Mm.	Mm.	Mm.	Mm.
Jan. 1.....	62	34	48.0	1.03	0.85	0.70	0.56
2.....	63	39	51.0	.93	1.43	1.25	.56
3.....	57	42	49.5	1.40	.91	1.00	.00
4.....	64	28	46.0	.93	1.07	.33	.00
5.....	64	32	48.0	.43	1.43	.66	.31
6.....	64	37	50.5	1.30	.64	.90	.50
7.....	64	33	48.5	.50	.00	.00	.00
8.....	60	43	51.5	1.00	.71	.25	.80
9.....	76	44	60.0	1.40	1.21	1.33	.30
10.....	80	47	63.5	2.20	1.43	1.16	.62
11.....	75	37	56.0	2.40	2.60	1.90	.94
12.....	77	39	58.0	3.40	3.70	1.90	1.56
13.....	65	48	56.5	4.30	4.00	2.00	1.30
14.....	69	29	49.0	3.40	4.00	2.80	1.30
15.....	55	36	45.5	2.30	4.40	2.76	1.30
16.....	50	34	42.0	2.14	1.86	1.83	.87
17.....	50	38	44.0	1.60	1.50	1.83	.81
18.....	46	33	39.5	.50	.64	.66	.00
19.....	49	44	46.5	.00	.50	.33	.00
20.....	56	34	45.0	.00	.43	.50	.00
21.....	60	32	46.0	.50	.00	.00	.00
22.....	58	33	45.5	1.20	1.30	1.16	.30
23.....	60	30	45.0	.14	.80	.75	.25
24.....	65	30	47.5	.50	.64	.75	.25
25.....	66	28	47.0	.50	.70	.93	.00
26.....	71	34	52.5	1.20	.84	.44	.50
27.....	70	34	52.0	.90	.72	.44	.50
28.....	66	31	48.5	1.26	1.10	.38	.40
29.....	70	32	51.0	1.80	1.50	.87	.80
30.....	69	33	51.0	2.10	2.00	1.20	.60
31.....	70	48	59.0	1.50	2.00	1.20	1.00
	° 63.6	° 36	° 49.8				

° Mean for month.

The tardiness of the Deglet Noor and Thoory trees in coming to full cessation of growth, with the fact that the Deglet Noor maintained some slight gain throughout the December period, suggests that if 50° F. is the zero point for the Zaheedy seedling, which represents the more tender Persian Gulf varieties, Deglet Noor and Thoory may have a zero point a little lower, possibly 48°; for it is to be expected that within the range of individuals and varieties of *Phoenix dactylifera* there may be a zero point difference of several degrees.

Again the thermograph sheet shows the actual time of exposure of these trees to the low maximum.

INDIO THERMOGRAPH RECORD, JANUARY 15-21, 1917

On January 15, after the temperature had been below 50° F. since 5 p. m. of the 14th, the maximum was 50° to 52° (maximum thermometer 55°) from 10 a. m. until about 1.30 or 2 p. m. From that time the temperature was 50° or below until noon of the 20th, giving a total as follows:

Summation, 50° or below:	Hours
January 15.....	10
January 16.....	24
January 17.....	24
January 18.....	24
January 19.....	24
January 20.....	12
Total.....	118

ZERO POINT RECORDS AT SAN ANTONIO, TEX.

Further evidence that the date palm zero point can not be above 50° F., and a hint that for some varieties it may possibly lie a little below that point, is found in the records secured on three seedling trees at the United States experiment farm at San Antonio, Tex., in March, 1917.³ Table III shows temperature records of standard United States Weather Bureau thermometers and corresponding daily growth of one leaf on each of three 8-year-old seedling trees for the first seven days of March.

TABLE III.—Temperature records and corresponding daily growth at San Antonio, Tex., of one leaf on each of three 8-year-old seedlings for March 1 to 7

[Critical low temperatures are indicated in boldface type]

Date	Temperature			Growth		
	Maxi- mum	Mini- mum	Mean	Tree 1	Tree 2	Tree 3
	°F.	°F.	°F.	Mm.	Mm.	Mm.
Mar. 1	74	44	59	20	18	14
2	53	41	47	6	4	5
3	59	41	50	3	4	3
4	46	32	39	3	1	2
5	50	22	36	2	1	1
6	61	31	46	2	2	2
7	80	46	63	1	1	1
8	70	38	54	5	3	1
9	73	39	56	5	5	4
10	69	58	63	9	6	4
11	84	64	74	21	11	13
12	81	67	74	22	12	15

After a maximum of 75° F. on February 28 (fig. 3) the thermograph trace crossed the 50° line at 4 a. m. of March 1 and remained at 4° to 6° below it until 10 a. m. of March 2. It passed above the

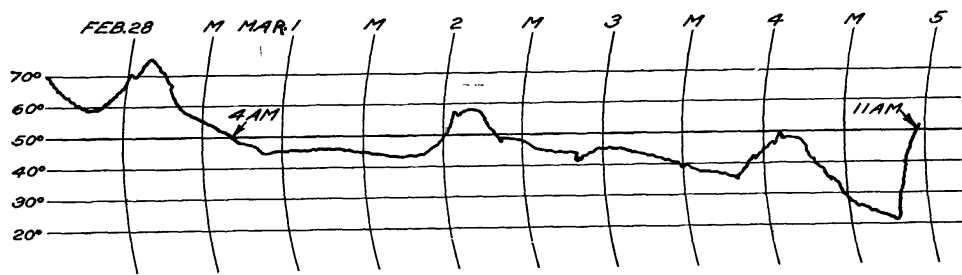


FIG. 3.—Transcript from a thermograph record from February 28 to March 5, 1917, at the United States experiment farm at San Antonio, Tex. The recording instruments were near the date palms from which the growth records in Table III were obtained

50° line at noon March 2, ranging up to 59°, and dropping below it at 8 p. m. It remained below 50° throughout the 3d and 4th, dropping to 22° at 7 a. m. of the 5th, but reaching 50° at 11 a. m.

³ For the careful keeping of these records the writer is indebted to N. H. Mercier, of the Office of Western Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture.

Summation of hours 50° F. or below of the thermograph record in Figure 3

	Hours	
Mar. 1, 4 a. m. to midnight.....	20	
Mar. 2, 12 midnight to noon.....	12	
Mar. 2, 8 p. m. to midnight.....	4	
Mar. 3, midnight to midnight.....	24	63 hours continuous.
Mar. 4, midnight to midnight.....	24	
Mar. 5, midnight to 11 a. m.....	11	
Total.....	95	

This makes a time lapse of 103 hours, of which 95 hours were below 50° F. and only 8 hours, on March 2, above this temperature. The depressing effect of this period reduced the daily leaf growth from 20, 18, and 14 mm., respectively, on the 1st of March to 2, 1, and 1 on the 5th and (after a characteristic lag) to 1 mm. each on the 7th; but it is to be noted that zero of growth is not completely reached even with this 95-hour exposure to a temperature below 50°. It must be remembered that these were seedling trees of unknown parentage, with the possibility of a lower zero point than those observed at Indio, and the fact of their failure to come to a completely dormant stage still leaves the absolute zero point for the species yet to be established. With the return to higher temperatures recovery was prompt, and the previous growth rate was surpassed on the 11th and 12th.

These widely dissociated and independent records of date-palm growth at Indio and San Antonio, all indicating that the lowest temperature limit for growth lies somewhere at from 48° to 50°, become so cumulative in value that only a laboratory test of the temperature at which growth will cease under prolonged exposure can add to their conclusiveness.

So long as maximum temperatures of 48° to 50° F. are accompanied on the same days by minimum temperatures from 5° to 18° lower, as has been the case on the crucial days at both Indio and San Antonio, it is not safe to affirm positively that the point of cessation of growth in the date leaves has not been in some measure influenced by the lower temperatures.

The fact that under field conditions growth has been made with similar or lower minima but with higher maxima makes the presumption a strong one that the zero point of growth is reached at a definite maximum temperature of the day, which becomes the minimum for cell activity. When laboratory conditions permit of exposing a plant to a very narrow range of temperature, the point at which growth ceases after a considerable exposure may be accepted without further question as the physiological minimum or zero point for growth of that plant.

Reference should here be made to the article by A. E. Vinson,⁴ who as early as 1914 made use of the 50° temperature point in studying date-palm growth, but did not, as is shown below, assume this as the actual zero point. As the Tempe Cooperative Date Garden growth measurements were made only weekly, they could not be coordinated with the air temperatures closely enough to secure a real zero point determination.

⁴ VINSON, A. E. THE EFFECT OF CLIMATIC CONDITIONS ON THE RATE OF GROWTH OF DATE PALMS. Bot. Gaz. 57: 324-327, illus. 1914.

Vinson, in analysis of weekly growth measurements made for Forbes at the Cooperative Date Garden at Tempe, Ariz., in 1906 and 1907, assumed an "empirical temperature" of 50° F., but on an incorrect hypothesis, for he writes:

For the present case 50° F. was selected (for the "empirical temperature, below which no marked growth takes place"). This is not to be construed as meaning that no growth would occur at a uniform temperature of 50° F., but under actual climatic conditions the minimum temperature which accompanies a maximum of 50° F. would effectively inhibit growth.

This last conclusion is proved erroneous nearly every winter, for maximum temperatures of 46° to 50° F. with minima above 32° are not rare in the date-palm regions. (See Table II, records of January 16, 17, 18, 19, in comparison with records for December 7 to 12 in Table I.)

During the period covered by Vinson's article the observed palms reached zero growth only during the first week of January, 1906. The official weather records for that time show: January 1, maximum 53°, minimum 29°; January 2, maximum 51°, minimum 19°; January 3, maximum 53°, minimum 18°; January 4, maximum 59°, minimum 20°.

The three days with minima of 19°, 18°, and 20° would alone account for the cessation of growth, leaving the real zero point undetermined.

ZERO POINT DETERMINATIONS UNDER LABORATORY CONDITIONS

Through the courtesy of Frederick V. Coville, botanist of the Bureau of Plant Industry, space was secured in a cool chamber which he had constructed in one of the Department of Agriculture greenhouses in Washington, D. C., where, by means of a small refrigerating machine, the temperature could be controlled within a range of from 3° to 5° F. Three seedling palms of the Thoory variety in 8-inch pots were selected for this work, and after a test of their activity at greenhouse temperatures (about 70° to 80°), were placed one or two at a time in the cool chamber. This cool chamber occupies the position of a greenhouse bench and is about 18 inches deep, 3 feet wide, and 20 feet long. A quantity of saturated sphagnum on its floor insured a rather even condition of high humidity in the air. The greenhouse roof above the bench is shaded during the greater part of the day, so that the light that reached the palms in the cool chamber was considerably reduced in intensity. The temperature of the chamber was recorded by a Richard thermograph, the accuracy of which was checked by a highly sensitive mercury thermometer placed near it.

The governing by the machine thermostat was somewhat erratic and gave a wider temperature range than was desirable. During the main period of 32 days (fig. 4) there was an extreme range from 48° to 56° F. But the time below 50° was only 1.2 per cent of the whole period and the time above 55° was negligible. The mean of the period was, as closely as can be estimated, 53°. The second period, covering five days in which readjustments of the temperature were made, gave a range of from 46° to 54°, the last 48 hours showing a mean of about 49°. During the third and last period of 48 hours, after the thermostat had been again adjusted to a lower temperature, the range recorded was from 44.5° to 49.5°. The peaks of highest

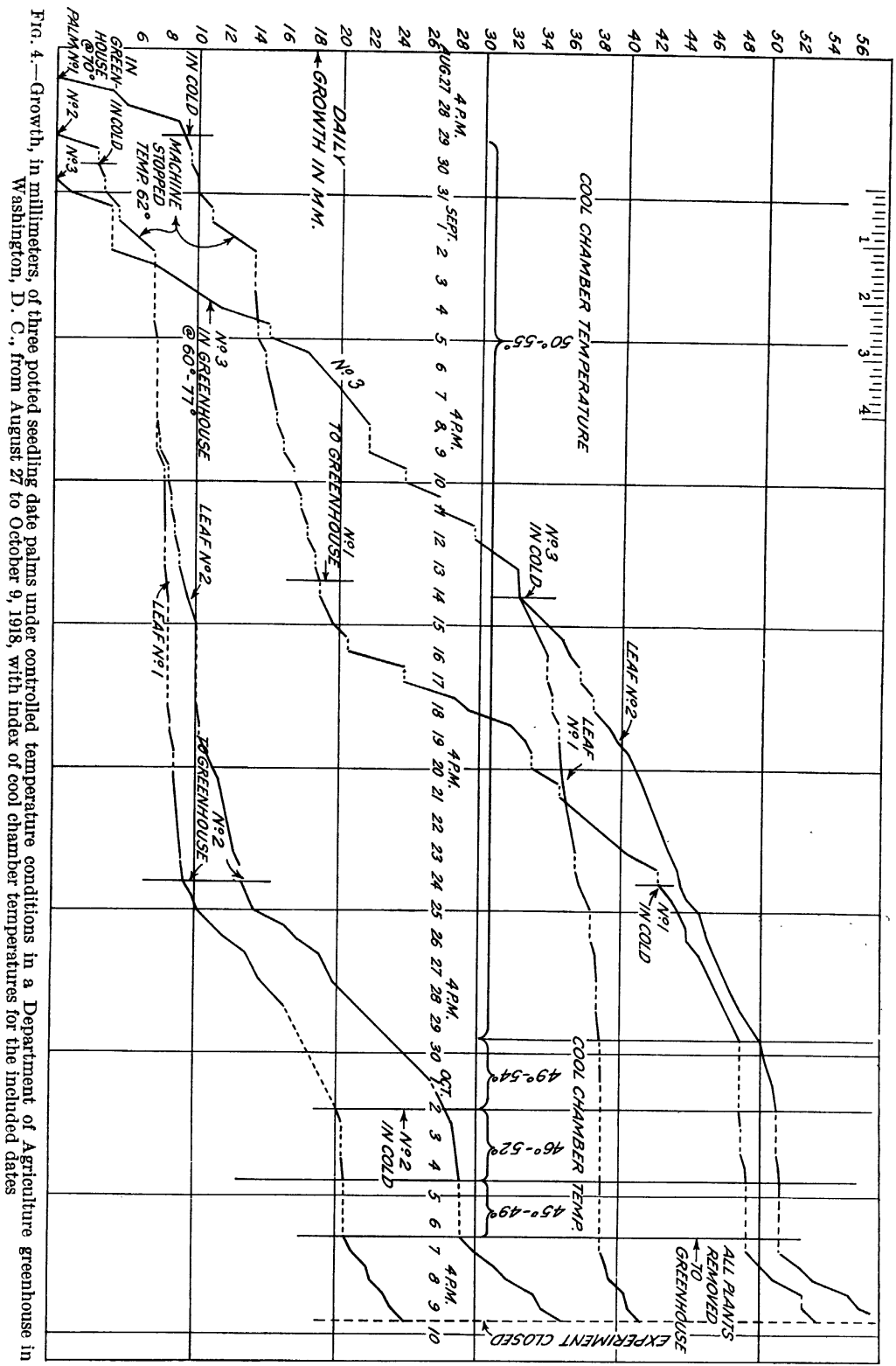


FIG. 4.—Growth, in millimeters, of three potted seedling date palms under controlled temperature conditions in a Department of Agriculture greenhouse in Washington, D. C., from August 27 to October 9, 1918, with index of cool chamber temperatures for the included dates

temperature for this period were quite evenly about two hours apart, the shape of the trace showing only slightly less time in the cooling operation than in the rise to the higher temperature at which the machine started again. The line of 47° , as the mean, divides this trace as equally as it can be done.

Since even in a cold chamber so shallow as this the difference in the temperature between the top and bottom air is considerable, these palms were laid down so as to bring them as nearly as possible on a level with the recording thermograph.

Though palms Nos. 2 and 3 were in turn placed in the cool chamber or used as controls in the greenhouse air, the history of palm No. 1 is so typical that its description will serve for all:

Palm No. 1, with a leaf in active growth, marked for record, was placed in air conditions in the greenhouse at about 70° day temperature and made 9 mm. growth in two days. It was transferred to the cool chamber at 4.40 p. m. on August 29, where, at a mean temperature of 53° , growth dropped at once to 0.5 mm. daily.

Except a short period when the machine stopped and the chamber temperature ran up to 62° , the growth averaged 0.45 mm. daily for $14\frac{1}{2}$ days. Palm No. 3, placed in the cool chamber September 14 under the same temperature range, made 17.4 mm. of growth in $15\frac{1}{2}$ days. This continuous activity under prolonged exposure to these temperatures so near the zero point is an important fact which could not have been disclosed under field conditions.

Palm No. 1 was next transferred to the greenhouse, where day temperatures were 70° to 77° , and made 1 mm. daily gain for two days, then the rate rose to 3 mm. daily. For 10 days under greenhouse conditions the mean daily gain was 2.4 mm. Again placed in the cool chamber September 24, with a temperature averaging 53.5° , this palm made a daily average gain of 1 mm. for the next six days. From noon of September 30 to noon of October 2 the temperature of the cool chamber was lowered to a range of from 49° to 54° , and from noon of the 2d until noon of the 5th it ranged from 46° to 52° . During the first two days no gain in growth was registered, but a gain of 0.6 mm. was made during the following three days at the lower temperature. With the maxima above 50° only six and one-fourth hours, or 8.7 per cent of this time, this close approach to the zero mark is very significant, as a similar reduction in growth was recorded for the two other plants which were in the cold chamber at this period.

Because of the wide range in temperature allowed by the thermostat, it is not certain at this critical period whether this slight growth should be credited to the brief periods when the temperature was above 50° or whether some part of it may have been made at temperatures slightly lower. During the next two days (from noon of October 5 until 9 a. m. October 7), with the temperature ranging from 44.5° to 49.5° , growth on all three plants entirely ceased. Here again the lapse of time during which the temperature was above 49° or below 45° was so small as to be negligible, but the time with the temperature above 48° was six and one-half hours, or 14 per cent of the whole period. This was time enough to have permitted a trace of growth had this been within the growth zone of temperature. By these tests, then, the positive exclusion of growth lies below 49° , with room for doubt whether any growth was made below 50° .

Thus the greenhouse and cool-chamber tests confirm the widely separated field observations in placing the zero point for the date palm at 50° F. or slightly lower. The time of exposure was sufficient for the entire plant (leaves, stem, and roots, with the ball of earth) to have been reduced to the cool-chamber temperature, and we can conclude that the physiological activities which lead to growth were suspended at that point. *The zero point for these palms, then, was the temperature attained by the growing center of the bud at which all growth ceased. We may assume that the same condition holds for large palms in the field.*

At the end of the cool-chamber experiment, after all three plants had been brought to a section of the greenhouse where they had full sunlight and a day temperature of 68° with night minima of 55° to 57°, palm No. 2 began growth during the day, the others the following night, and made gains of from 3 mm. to 8 mm. on the different leaves during the next three days.

These figures clearly show that the plants had sustained no injury by being brought to the zero point of growth. With a slight time allowance necessary to bring the roots in the mass of earth in the pot to the new temperature, they were ready to resume normal activity when exposed to the higher temperature.

With the zero point for constant exposure known, the next step is the application of such knowledge to field conditions, subject to the varying ranges of daily temperature.

Daily mean air temperatures of 50° F. seldom bring the date-palm growth to zero. At Indio a slight growth has been recorded under mean temperatures for weekly periods as low as 45°, but these were where the component maxima were in the 60's and 70's and frost minima were sustained for but brief periods.

At Eureka, Calif., with but few frosty nights and minima rarely below 28° or 30° F., but with monthly means of from 46° to 56° F., giving a normal annual mean of only 51.3°, the growth of the date palm is entirely excluded. At San Francisco, nearly frostless, with monthly means from 49° to 62° F. and the normal annual mean only 54.9°, the date palm makes a slow growth, but seldom, if ever, flowers.

For a comparison of the relative temperature efficiency for the vegetative growth of the date palm of localities not subject to destructive minimum temperatures, if the mean of 50° is taken as the base the monthly summations above that point during the growing season will afford a reliable scale of values.

SUMMARY

The date palm, *Phoenix dactylifera*, as represented by its horticultural varieties, has a wide range of temperature endurance, being able to survive without permanent injury temperatures in different localities from 4° to 125° F.

The geographical range of the date palm as a plant is very much wider than the range of its successful fruit production, and the cultivated varieties are often rather exacting in their temperature requirements.

Date trees, under favorable environment, continue growth throughout the year at a rate closely correlated with the current mean

temperature. Growth may continue at a reduced rate when the minimum air temperatures are several degrees below the freezing point, provided the maximum temperatures of the days are well above a minimum of 50° F.

Observations at the United States date garden at Indio, Calif., show that with minimum temperatures above the freezing point but with the maxima of the days reduced to from 46° to 50° F., growth of four date palms, after a characteristic lag, has been found to have entirely ceased. A specific zero point or minimum of temperature for growth of between 46° and 50° F., but varying slightly with the individuals, has been deduced from these records.

Young date palms in pots placed for 14 days in a controlled temperature bench in a greenhouse at a temperature varying but slightly from 53° F. made daily growth of from 0.5 to 1 mm., and slight gains were recorded at from 49° to 50°. Below 49° all growth ceased. The plants experimented with in the greenhouse cool chamber when placed in greenhouse benches at day temperatures of about 68° F. resumed their normal rate of growth in darkness without appreciable delay. The laboratory tests thus confirm the previous field observations.

The conclusion is reached that the zero point, or minimum temperature permitting, growth of the date palm, lies at from 48° to 50° F. for the actual region of cell division and growth, and that this knowledge is fundamental to the study of the reactions of these trees to other temperature conditions.

PARTIAL THERMOSTASY OF THE GROWTH CENTER OF THE DATE PALM¹

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INTRODUCTION

The date palm (*Phoenix dactylifera*) is the most important member of the great palm family, excelling even the coconut palm in its economic value to the human race. Its culture is one of the most ancient of which we have records, being recognized in Assyrian tablets of very ancient date, and possibly even earlier in architectural decorations in Egypt. Soon after the children of Israel had escaped from Pharaoh across the Red Sea we are told in Sacred Writ² that "they came to Elim, where were twelve springs of water and threescore and ten palm trees: and they encamped there by the waters." The springs are there to this day, and some palms. The place is called in the Arab tongue "Ain Musa"—"The Springs of Moses."

In its climatic requirements the date palm is in strong contrast to the coconut palm, which finds favorable environment along tropical reefs and shore lines having abundant rains and an almost saturated atmosphere, yet without excessive temperatures, and which is tolerant of only the very lightest frosts. The date palm, though fruiting in some coastal regions like northern Egypt, requires a practically rainless season for the perfect development of its fruit, and is at its best in hot interior regions having very high temperatures and low humidity. The practical culture of this palm in the northern hemisphere extends southward to about 15° latitude, but ceases where the region of tropical rains begins. In west Africa the commercial production of dates is confined to the region on the south side of the great Atlas range, although beautiful specimens of non-productive trees may be seen at Algiers and other Mediterranean cities. A small but very significant commercial culture of the date palm is maintained at Elche, in the southeastern part of the Spanish Peninsula, at a latitude just above 38°, the most northerly point of commercial date culture in the world. Nonfruiting trees may be seen at most points along the northern Mediterranean coast, even extending to Venice at 45° on the Adriatic, but fruit production reaches only a latitude of 35° in Mesopotamia, 34° in Persia, and about 30° to 33° in the Punjab in India.

In the United States, to which the cultivated varieties of dates of Africa and Mesopotamia have been transplanted, fruit has been successfully matured only as far north as 30° to 33° in Arizona and about 34° in California, with possibilities of commercial development as far north as 36° or even 38° in the interior valleys. These data show the approximate range of date culture in the northern

¹ Received for publication September 29, 1924; issued October, 1925.

² Exodus xv. 27

hemisphere; and indicate the range of temperature and humidity which may be encountered.

Temperature and humidity ranges in the date-growing region of the Nile Valley, from Alexandria and Rosetta on the Mediterranean coast to Khartoum, comprising a practically continuous north-and-south extension of this culture for over 1,000 miles, were shown in diagrams and discussed by the writer in 1914 (16).

Port Said and Alexandria represent in their monthly means of from 56° to 80° F. about the lowest temperatures at which commercial date culture may hope to succeed, here accompanied by high humidity, with monthly means of from 64 per cent to 76 per cent throughout the year. Merowe and Atbara in Sudan have nearly the upper temperature limits at which date culture may be conducted, with monthly means of 68° in January and 93° in June. The relative humidity at Merowe is the lowest recorded for a date-producing region, with means on a 15-year record of only 12 to 16 per cent from March to June and an annual mean of 22 per cent.

But even with the great range of adaptability as seen in Egypt, the full measure of adjustment to temperature of which this tree is capable is not attained. In the Salt River and Gila River Valleys of Arizona date palms have survived minimum temperatures of from 9° to 11° F. with the loss of only the exposed leaves, then, pushing up new leaves promptly with the return of warmer weather, have produced abundant crops of fruit in the year following. Nonfruiting seedling date palms in San Antonio, Tex., have survived minimum temperatures of 4° F. with continuous temperatures below the freezing point for more than 48 hours. At the other extreme, maximum temperatures of 121° at the United States Date Garden at India, Calif., have not seriously impeded the growth rate of the leaves of young date palms nor impaired the development of their fruit.

Such facts regarding a tree of the first economic importance invite the closest study of any peculiarities in its anatomical structure and physiological action which may enable it to endure such vicissitudes. The Arab's idea of the wants of the date palm, "Its feet in running water, its head in the fire of the sky," has so often been repeated, that the growing of the tree under such conditions has been accepted as a matter of course. No one seems to have been aware that the widespread, powerful root system, the sturdy and lofty trunk, capped by the single giant bud with its deeply seated growing point, and majestic crown of a hundred leaves, together comprise a remarkable provision of nature for the protection of the embryonic cell tissues against wide extremes of both heat and cold, and furnish a stabilizer of growing-point temperatures for this tree of the desert.

Like all palms, the date palm is endogenous, its growth internal, with no sensitive cambium zone to be exposed to sunscald under the desert heat. Like all but a small group of palms, it has an unbranched cylindrical trunk, the product of the perennial intercalary growth of a single terminal bud, which in this species is of enormous size.

DeCandolle (5, p. 37) had the impression that the date palm has a distinct dormant or resting period after its fruit is matured and during the winter months, and he speculated concerning the tem-

³ Reference is made by number (*italic*) to "Literature cited," p. 462.

perature under which growth would be resumed in the spring. Brown (4, p. 65) also assumes a dormant period for this tree, and says: "The vegetative growth of the trees commences toward the end of April or the beginning of May * * *."

It has been left to later observers, however, to prove that the date palm in favorable habitats has no resting period, but with water supplied to its roots, continues to push up leaves from the growth center throughout the year. Several complete years of observation show that only minimum day temperatures below 50° F., or destructive night temperatures of 21° or 22° will bring this upward growth to a temporary standstill (17).

THE AVAILABLE DATA

Two series of observations on the growth rate of date palm leaves through two full years have been kept in the United States. The first, a condensed report of which was published by Vinson (24), was made at the Cooperative Date Garden at Tempe, Ariz., including the years 1906 and 1907, under the supervision of R. H. Forbes, Director of the Arizona Experiment Station. These growth measurements were made weekly. The second was a series of daily growth measurements, kept in part by the writer and completed under his direction, begun in October, 1916, and continued through the full years of 1917 and 1918 at the United States Experiment Date Garden at Indio, Calif. An analysis of these records is not in the province of this paper, but there is a striking agreement in the results obtained, considering the remoteness of the two stations, the difference in the ages of the trees studied, and in the soil and water conditions.

Both records agree as to two important facts in the activity of the date palm: (1) Except for very brief intervals under exceptional conditions, growth is continuous throughout the year; (2) the growth curve, which took no account of volume but was a record of the upward growth of the more active leaves of each tree, follows a course generally parallel to the curve of weekly mean temperatures; the growth is very slow when daily means are below 50° F., and the most rapid growth follows the highest means of 90° to 100°.

In Figures 1 and 2 the mean weekly growth and mean weekly temperatures for 1917 and 1918 are plotted on the basis of zero of growth at a mean of 40° (unity at 42°) and a gain of 1 mm. in mean growth for each gain of 2° in mean temperature.

Plotted on this scale the curve of growth rate falls below the heat curve until about the first or the middle of March; holds increasingly above it from April until midsummer when the peak of the growth rate is reached, and continues above it through a mild autumn to the end of the year.

A decline from 65.5° to 53.5° in the latter part of November, 1917 failed to bring the growth curve down to meet the temperature curve.

As for going into a dormant state, as was assumed by DeCandolle, the average growth rate was still 10 mm. daily and with the unseasonable rise of the temperature means to 64° at the end of December, the mean growth rate for the first week of January, 1918 (fig. 2), advanced to 14 mm. daily. The decline of the January, 1918, temperature means from 61.5° to 49°, however, caused a

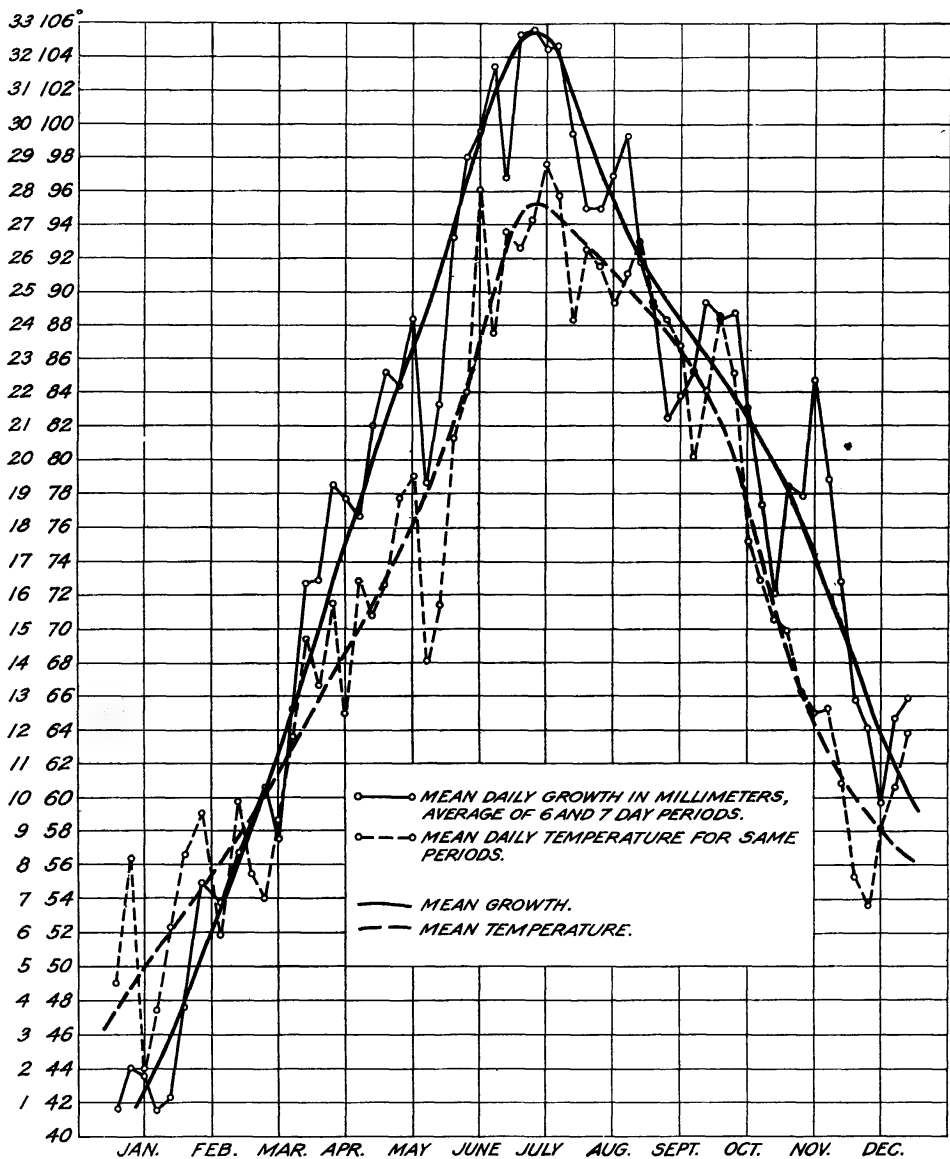


Fig. 1—The relation of the daily leaf growth of four date palms at the United States Date Garden at Indio, Calif., throughout the year 1917 to the daily mean temperature. The temperature is plotted in 6-and 7-day periods and the mean growth in millimeters for corresponding periods on the basis of zero of growth at a mean of 40° and a gain of 1 millimeter in the daily growth rate for each rise of 2° in the daily mean temperature

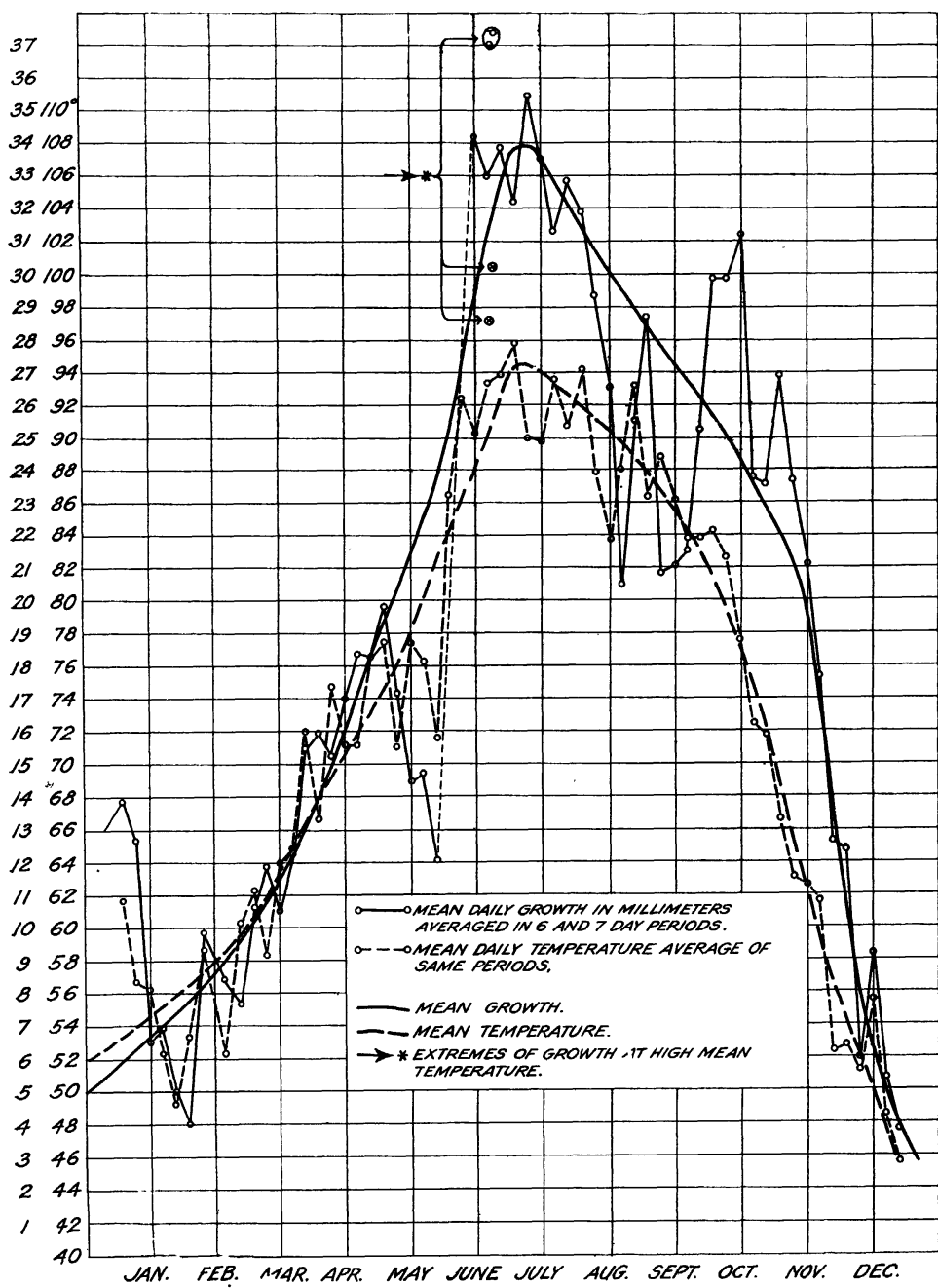


FIG. 2—Corresponding records of growth and temperature for the year 1918. (See fig. 1)

check in the daily growth rate from 14 mm. to 4 mm., but with a quick rising when a temperature mean of 60° was gained, showing the tendency to a perennial growth when the temperature permits.

In other words, though growth in the date palm is sustained throughout the year under Indio and also Tempe temperature conditions, growth is proportionally more rapid at the higher than at the lower temperatures. It will be noticed also that fluctuations of 10° to 20° in the mean temperatures are rather closely followed by corresponding fluctuations in the growth rate, though there are some startling exceptions to this rule in the late autumn growth of both years.

The conditions under which leaf elongation in the date palm is brought to zero are as striking as the fact of its practically all-year growth. While with ordinary plants growth is so nearly confined to "the average frostless season" that "climatic zones" are usually based on such seasonal lengths (12) the date palm may show increment in leaf growth when minimum temperatures fall below the freezing point for several days in succession. For example, for three consecutive days at Indio in December, 1916, with minima from 24° to 21° F., growth was not wholly checked when the maxima of those days were above 60°. On the other hand, it was observed twice at Indio in the winter of 1917 that with the minima above the frost line, but with the day's maxima falling below 50°, growth was brought to zero (17). In general it may be affirmed that the growth rate in the date palm is more closely coordinated with the temperature throughout the year than that of any other plant species yet studied.

Repeated field observations made by the writer, supported by extended records of growth under controlled temperatures, have demonstrated that the minimum temperature permitting growth of the date palm is close to 49° or 50° F., provided this temperature reaches the growth center (17). This zero point of temperature for growth probably varies slightly with different varieties and possibly with the individual tree, growing under different conditions. The zero temperature for growth is rarely reached under the climatic conditions prevailing in date-producing regions, and then only for very short periods.

It is evident that the mode of growth of the date palm is in striking contrast to that of ordinary exogenous fruit-bearing trees, such as the apple, peach, and fig.

Gourley (8) found that the total twig growth in a New Hampshire apple orchard under observation in 1916 was practically made during 35 days, from about May 25 to June 30, with a slight gain for 27 days longer. During this time the total daily growth on the observed twigs, computed in sixteenths of an inch, declined from 120 to 10, while the mean daily temperature advanced, measured by a smoothed curve, from about 55° to 75°; yet increase or decrease in daily temperature was followed more or less closely by corresponding changes in growth rate (fig. 3).

The year's twig growth of the apple orchard, chiefly made in a twelfth of the year's time, was in part the expression of the food-building capacity of the last year's leaf crop, and bore only an indirect relation to current temperatures. Increment on a single tree would have been distributed among a network of roots, hundreds of twigs

with thousands of leaves and buds, and a paper-like shell, a veritable cast of the complete tree would have been thrust between last year's bark and wood. Thus the twig growth, in length, was nearly all made in a single month; the addition of the woody shell continued for possibly four months.

DATE PALM STRUCTURE

In contrast with this typical exogenous tree, where the function of cell multiplication and growth is distributed through the cambium zone with its thousands of terminal buds, that of the date palm trunk is concentrated in the single giant terminal bud, or phyllophore,⁴ which at the same time is pushing up six or eight great leaves by

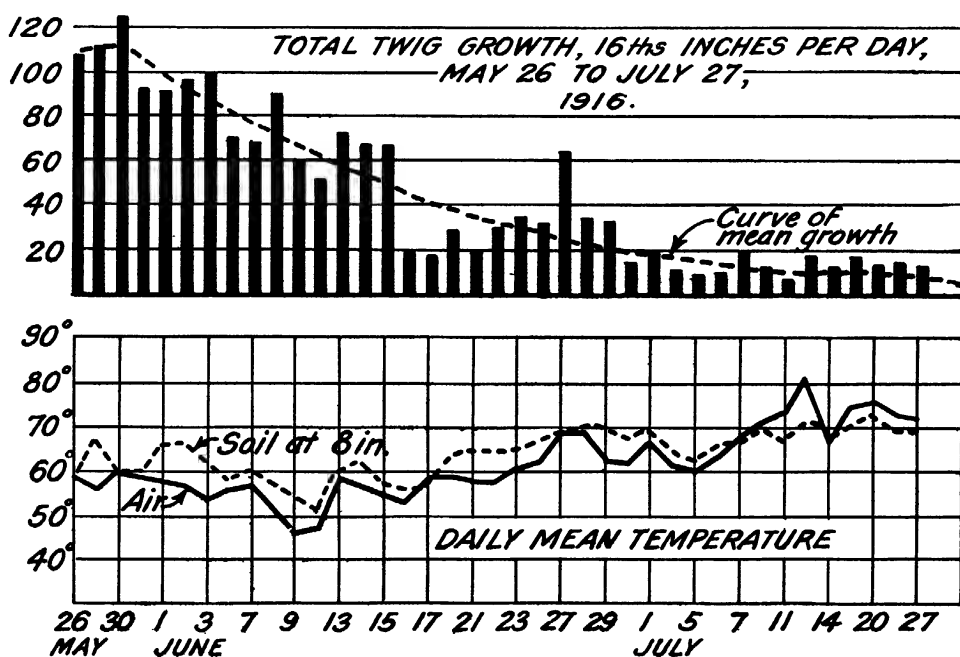


Fig. 3—Total twig growth for the season on selected trees of a New Hampshire apple orchard; below—mean air and soil temperatures for the corresponding period. The twig growth declines from the maximum to zero while the temperature advances

intercalary growth at their bases, laying down flower spathes in their axils for next year's crop, elongating the trunk from 12 to 18 inches a year, and bringing the trunk diameter in the tapering "cone of growth" up to the pattern set by the shaft below. Except at the growing tips of the multitude of ropelike roots, each pushed independently from the spherical base of the trunk, the growth area of this palm is close to the base of the crown of giant pinnate leaves, where the work of photosynthesis is performed and where, under the intense light and dry air of desert conditions, the large volume of

⁴ "Phyl'ophor, Phyl'ophore, *Phylloph'orum*, (Φορτω, I carry), the budding summit of a stem on which leaves are developing, especially applied to palms." JACKSON, B. D. A GLOSSARY OF BOTANIC TERMS. Ed. 3, rev. and enl., 427 p. London. 1916.

Apparently this term was first used by Mirbel (18) in a communication to the French Academy, *Comptes Rendus*, 12 Juin, 1843, p. 1216, from which is quoted the following: "Quand on a terminé ce travail, la structure de stipe devient aussi claire que d'abord elle paraissait obscure. En voici la raison: le bourgeon ne peut se développer qu'autant que de nouveaux filets pénètrent dans le phyllophore et se dirigent vers les jeunes feuilles."

Martius (15, p. LXXIV), who was contemporary with Mirbel and familiar with his work, notices the use of this term as follows: "Illum locum, quem Germani botanici vocarunt gemmae nucleum 'Knospennkern,' novo nomine insignivit Mirbelius 'Phyllophorum'."

water is transpired (pl. 1). This juxtaposition of the region of photosynthesis with the region of cell formation and growth in the date palm has a very important relation to its general activity.

"Centralization of activities" is the keynote of date-palm growth. Its leaves, usually 8 to 12 feet long, are not, as is the case with many palms, loosened from the trunk with maturity, but the bases are strongly attached by tough fiber bundles which penetrate deeply into the cortex. From 10 or 12 to 20 leaves are pushed up from the center of the crown each year. These retain their efficiency four or five years, but gradually turn yellow and dry up, when they are pruned down to within 12 or 15 inches of the place of attachment by the date grower.

The structure of the date-palm leaf is of the greatest importance in securing the insulation of the growth center. The rachis or midrib at the base of the blade, arbitrarily assumed to end at the lowest spines, may be 1 to 2 inches in diameter. From this point the lower portion, considered as the petiole, broadens and thickens more or less rapidly according to the variety and age of the plant. In mature leaves the base may be from 8 to 12 inches broad at the attachment to the trunk, and from 1 to 2 inches thick in the center, thinning out in the lower portion to the marginal thickness of the clasping sheath.

Each leaf petiole has a triple-layered cylindrical sheath from 12 to 20 inches long, at first white and crisp as lettuce leaves, but becoming a tough sheet of diagonally-crossed brown fibers upon expansion and exposure to the light. The line of attachment of the lower margin of the sheath to the trunk marks the beginning of the very short internode of the palm trunk. As the succession of these ribs and sheaths is developed from within, six or eight concentric layers may encircle the phyllophore and their combined strength binds the upper spirals of leaves firmly together⁵ against the leverage of the winds on the broad leaf blades. These sheaths, with the persistent heavy fibrous bases, form a most efficient insulating and protecting layer surrounding the delicate meristematic tissue of the growing plant (pls. 2, 3, and 4).

With the crowding up of new leaves in the center the sheath is finally torn from the sides of the base of the petiole, but the successive sheets, attached to the trunk by their lower margins, are tightly wedged behind the encircling bases, 13 nearly vertical ranks of which may be counted around the trunk. In the dry climate of Indio, Calif., and other date growing regions, these bases and sheaths usually persist for many years, covering the entire trunk with a thick mat of dry fiber interspersed with air spaces, and becoming a protective layer.

Plate 1 gives an excellent idea of the external appearance of this protective layer; but Plate 3, a cross section of a date-palm trunk about 2 feet in diameter, shows very beautifully the imbricated arrangement of the spiral succession of leaf bases; while Plate 5, shows how effectively these layers insulate the tender tissues of the phyllophore.

⁵ It may occasionally happen that the sheaths bind the apex of the bud so tightly that the new growth of leaves is unable to force its way out.



A general view in the Cooperative Date Garden (University of California and United States Department of Agriculture) at Mecca, Calif. Trees about 15 years old, in full bearing. Tree in right foreground a Deglet Noor. Note the method of encircling of leaves and fruiting stalks from the plant terminal bud and the protective envelope of leaf bases and sheath fiber or "leaf" inclosing the trunk.

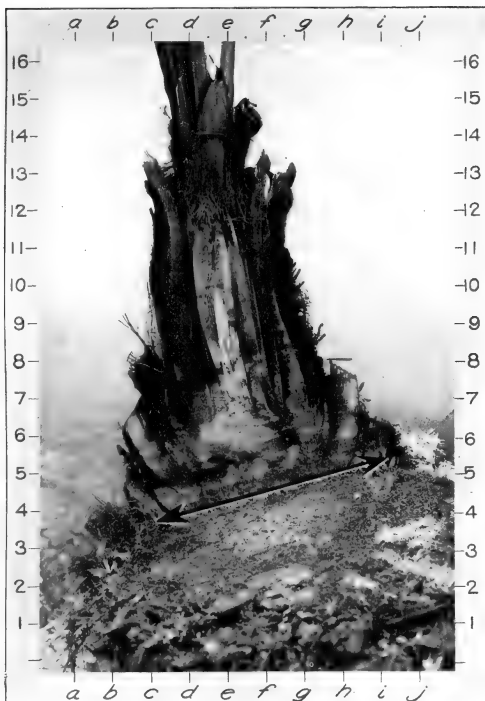


Top of 10-year-old seedling date palm at Santa Barbara, Calif., which was sectioned to secure plates 3, 4, and 5, showing above *A* a group of newly projected leaves with the pinnae still tightly folded fanwise against the rachis



A cross-section of the trunk of the same date palm shown in Plates 2, 4, and 5, about 23.5 inches in diameter. (See white 10 cm. scale.)

The trunk proper, stained with potassium iodide to show the starch content, is surrounded by the imbricated leaf bases, 7 to 8 inches broad, which form a highly nonconducting protective layer



Above arrow, longitudinal section through the heart of the date palm shown in Plate 2; in front of arrow, horizontal section of half of palm

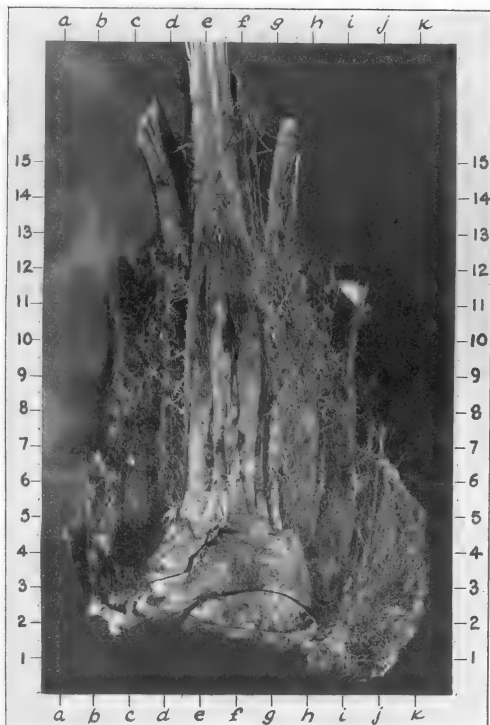
Print mounted on square ruled paper, ordinates 1-17, abscissa a-k

6 to 6.5 *e* to *e* 7, growth center from which group of leaves has sprung by basipetal elongation. 6.5 to 10, with bases *e* to *e* 7, longitudinal section of group of very young leaves. They are pushed upward by growth made wholly within the bud

6.5 to 11.3, breadth *d* 1 to *f*, longitudinal section of bases of group of leaves 4 or 5 feet high, with pinnae still unexpanded, cut tops seen at *e* 5 to *d* 3, also in Plate 2 above A. These are making their most rapid growth

8 to 13, breadth *c* 5 to *g*, longitudinal section of bases of older leaves, still making active growth. 5 to 6.5, breadth *d* to *f* 5, very tender, white brittle meristem, giving quick starch reaction. This is the "heart" of the palm that is sometimes eaten. It is at once the growth cap of the trunk and the source of supply of all leaves still making basipetal elongation

2.4 to 6.8, opposite diagonal line showing base of longitudinal section of trunk. In front is the horizontal section of the stump, also showing active starch reaction



The longitudinal half of the palm removed to secure Plate 4, photographed in studio an hour later on a larger scale

The area 2 to 4.7-c 5 to *h*, is the upper portion of the phyllophore, a mass of meristem, ivory white, and very brittle in spite of the formation of the fibro-vascular bundles, which are here clearly shown, owing to the oxidizing in the air. This is forming the trunk elongation and thickening below while pushing up the new leaves from its apex. The group with their bases at 4.7, in breadth from *d* 5 to *g* 3, are in active elongation, their tops showing above 4 in Plate 2

Their active area of elongation is probably from 5 to 7, but in the region from 8 to 12 the bundles are already well formed, preparing the leaf for exposure to the wind, and for its active service in transpiration and photosynthesis

The growth characters of the date palm may be summarized thus: In the seedling a bulb-like stem pushes up from its growth center by basal increment centripetal spirals of pinnate leaves which make no elongation after they emerge. From the rounded base of this stem is produced an increasing number of cordlike roots which may ultimately reach the thickness of a finger and extend over a radius of 30 to 40 feet or more. They have only small lateral branches and feeding rootlets without root hairs. These roots must convey a large volume of water to the trunk, and at the same time are so provided with fiber bundles that they can support the increasing height of the trunk against the pressure of the wind.

While there is a rapidly increasing production of leaves from the crown until normal development is reached, trunk growth for the first few years is mostly absorbed in increasing the diameter to the size characteristic of the variety, usually from 20 to 30 inches. As the tree advances in height this diameter increment is in progress over a considerable region, so there is always an apical "cone of growth" of varying lengths in different varieties, usually 3 or 4 feet, within which tissue building goes on.

Unlike the old wood or duramen of an exogenous tree in which the sap channels soon become filled up and unable to convey the upward current, the vessels of the date palm trunk remain open and able to convey a strong flow of sap to the crown of transpiring leaves throughout the long life of the tree.

The apical portion of the phyllophore is in a continuous state of subdivision into leaves, which are at the same time pushed to the outside by basipetal elongation and by the upward growth of the central axis. The new leaves are provided with fiber bundles, apparently by the branching of the bundles from below, the main axis continuing in the vertical direction.

We must conceive of growth of the bundles taking place something as in the diagram (fig. 4). The basal portion of the leaves remains in a state of cell division and elongation for many weeks, while the blade as it emerges to the light quickly develops chlorophyll and takes on the characters of permanent or somatic tissues, able to endure the vicissitudes of its surroundings. There is soon established also a differentiation between the leaf tissue and bole or trunk tissue, for while date palm leaves usually retain this attachment to the trunk for a long time, a cross section at any time 2 or 3 feet below the top of the bud shows a well marked difference in structure between the leaf tissue and the trunk tissue. In the physiological action described in this paper a clear distinction must be kept in mind between (1) the protecting envelope comprising the broad bases of the midrib, with the enveloping sheaths, and (2) the trunk proper. These are shown admirably in Plate 3, a cross section of the same palm shown in Plates 4 and 5, at 2 feet above the ground.

There has been much speculation in earlier periods as to the mode of growth and of the development of the fibro-vascular bundles in palms and other plants of the monocotyledonous class, which led to the preparation of papers by Desfontaines, Hugo de Mohl, and other botanists in the latter part of the eighteenth and the early years of the nineteenth century; and in 1839 Mirbel was sent to Algeria by the French Academy of Sciences to study palms in detail. Several of his papers appear in the volumes of *Comptes Rendus* in 1843-44

and are discussed by Von Martius (15). The whole subject was reviewed at length by John Casper Branner (2) in 1883, and the results of his own researches in South America given, in comparison with these early theories. Three points in his conclusions have a distinct bearing on the subject of this paper:

(1) This author states (2, p. 467):

I would suggest that this development of the fibro-vascular bundles, always in the direction of the apex is due to the light. This apex, at its central point, is very pulpy and translucent while its sides are enveloped in the young and growing fronds which render the parts surrounding the center more or less opaque.

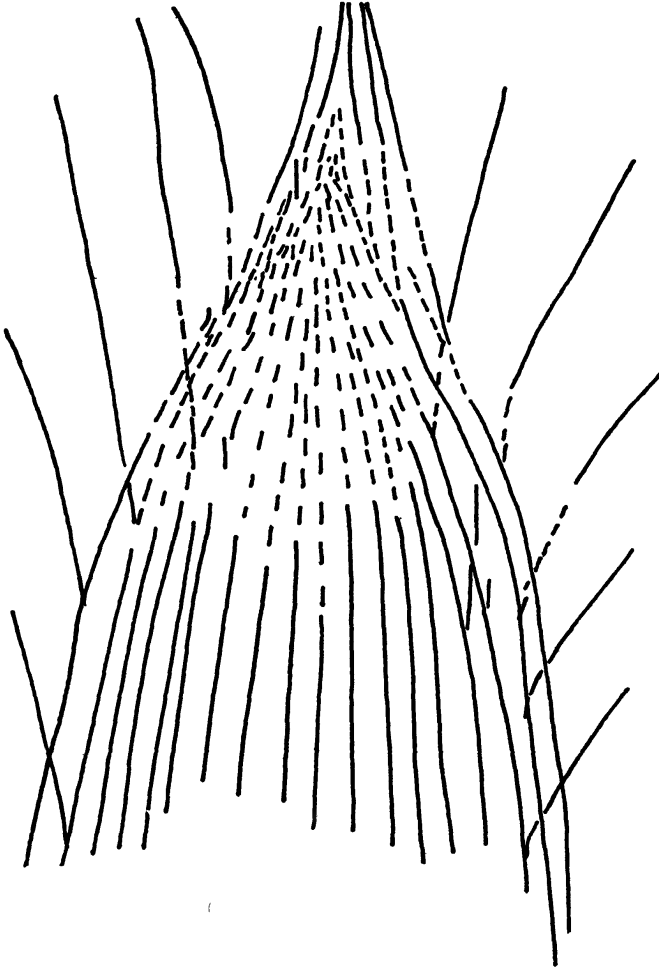


FIG. 4—Diagrammatic representation of the fibro-vascular bundles in the phyllophore of a date palm. Solid lines showing areas in which the bundles have completed their elongation; broken lines, meristematic areas in which the bundles are in process of formation and elongation with branches sent out to the new leaves

Reference should here be made to Plate 5, which shows the reverse half of the palm top cut from that portrayed in Plate 4.

This shows how impossible it would have been for any light to penetrate to the center of the cell division which would have been most active in the area between 4-7 and 1-g of the photo scale. The area 2-4 + d-h was, at the time of cutting, ivory white and so brittle that it cracked, as is shown, with the most careful handling. It had been exposed to the air about an hour when photographed and

the oxidizing brought out the tender fibers in strong relief. The early preparation of the leaf to resist exposure to the wind would require this formation of the embryo bundles within the zone of cell division where complete absence of light prevails.

Gradual exposure to the light as the group of leaves is pushed upward would be followed by the completion of the thickening and hardening of these bundles and the full development of the water-carrying tissues, but without gain in length.

(2) Branner also found (2, p. 470) that the total number of bundles was about uniform throughout the lower and upper portions of the trunk and that while branches were being continuously given off for the formation and support of new leaves, the number of bundles continuing into the main axis remained substantially uniform.

(3) Branner further states (2, p. 476): "A palm trunk may grow laterally as long as the fibro-vascular bundle divisions of the given part are in connection with active fronds." This in practice means that the cone of growth coincides with the altitude zone of active leaves, which is pretty well confirmed by field observations in California and Arizona.

OFFSHOOT BUDS AND FLOWERING BUDS

Morphologically, every date-palm leaf should produce in its axil either a vegetative bud or a fruit bud, though many fail of more than rudimentary development. The earliest buds on seedlings are invariably vegetative buds, giving rise to offshoots so low on the trunk that they often may become self-rooted in the soil. Offshoot buds may continue to be produced for many years, especially in damp coastal climates, but they usually are replaced permanently by flowering buds after a few years. These flower buds may be produced in an almost unbroken succession, but with a heavy production of fruit by the female trees one year, few or no fruit spathes may develop the next year. The staminate and pistillate flowers are borne on separate trees.

The fruit spathes always arise within a few leaves, often only six or eight, of the bud center, and usually develop in centrifugal order, the highest and youngest first, then the next older, spirally downward and outward. All are in closest proximity to the growth center, and the growth of the fruiting stalk is basipetal, as is that of the leaves. Frequently the lower and later flowering heads, though really the oldest in point of origin, seem to be one side of the most active life current and fail to receive the nourishment to bring them to full fruition. This is especially true in the case of male palms, where the late flowers are often abortive.

The interior substance of the young date-palm trunk, when tested has always shown the reaction for starch. To what extent this is a storehouse of reserve food is a question for further study.

The foregoing rather extended account of the structure and habitat of the date tree has seemed necessary to an understanding of the unexpected and remarkable temperature reactions which were observed on inserting thermometers into the heart of the palm trunk—some into the lower trunk and others into the actual growth center.

FACTORS GOVERNING THE INTERIOR TEMPERATURE

The temperature within the massive trunk of a date palm and the phyllophore must be governed by:

(1) The temperature of the surrounding air; (2) the loss or gain of the air temperature in translation through the leaf bases and trunk tissues to the interior; (3) the temperature of the soil at the depth of the feeding roots, assuming that this governs the temperature of the soil moisture as it enters the rootlets and as it traverses the roots to enter the trunk; (4) the loss or gain in the temperature of the ascending sap current in its progress through the trunk to the leaves; (5) an amount, difficult to estimate, of heat generated by the cell activities in the growth center—the heat of respiration.

The factors (1) and (3), both indirectly the products of the air temperature, affect the growth center quite differently; at different hours of the day and during different portions of the growing season. Hence the influence of each should be kept quite distinct in considering the results which follow.

There must also be considered here a factor whose influence we are wholly unable to measure, the cooling effect of the transpiration of the large volume of water from the leaf surfaces. The effect of this in the regions of photosynthesis is undoubtedly considerable. The influence of the return sap current bearing the products of photosynthesis on the general temperature of the phyllophore would be impossible to estimate, but it is probably negligible.

EXPERIMENTAL DATA

In the analysis by the writer of a series of observations on the rate of leaf growth of the date palm in relation to temperature some very significant points were brought to light. It was noted that when the maximum air temperatures were well above 50° F., not only was growth continued after a minimum temperature of 32° was reached, but at temperatures considerably lower; air minima of 25°, 24°, and even of 21° not wholly checking growth on fairly mature trees, though acting more severely on young seedlings. The inference arose that the growing center of a date palm bud must be in some way protected against the actual minimum of cold registered by a thermometer in air near it, unless the cold period is considerably prolonged.

Thermograph records show that minimum temperatures in date-growing regions of California and Arizona last but a few minutes. Shreve (22) concludes:

A consideration of the factors which have to do with the distribution and activities of the giant cactus (*Carnegiea gigantea*, *Cereus giganteus*) led me to believe that the greatest number of consecutive hours of freezing is the most important climatic datum in determining its northward range, * * *.

While with unicellular plants, as yeast, for example, variations of temperature to which the multiplying cells are exposed may be determined by a thermometer immersed in the culture medium, with most plants which develop tissue systems there is no opportunity to insert a thermometer in the growth centers without such destruction

to cell action as would defeat the end in view, consequently the surrounding air and the soil in which the roots are growing must be relied upon to furnish such determinations.

Fortunately the trunk of the date palm, with its single enormous terminal bud, within which all leaf growth and elongation takes place, affords ample space in which to place a thermometer without serious disturbance to the functions of the plant.

For a study of interior temperatures by this method, a Zaheedy male seedling,⁶ one of the trees upon which leaf growth records have been kept at the United States Date Garden at Indio, Calif., was first selected. It had a trunk height of 5.5 feet and a diameter (outside of the leaf bases) of 18 inches at 2½ feet from the ground. At 4 feet from the ground, or 18 inches below the top of the bud, its diameter was 13 inches. Two ¾-inch holes were bored to the center of the trunk, one 18 inches below the apex of the bud, and one on the opposite side of the tree at 30 inches from the ground. A 13-inch chemical thermometer was inserted in each hole after being pushed through perforated corks to close the holes and yet permitting the bulbs to reach the center of the trunk. The bulbs were covered with sleeves of thin rubber tubing to prevent too quick a change in the readings. A third thermometer was installed in a frame close to the trunk of the tree and screened so as to give practically the conditions of a Weather Bureau shelter. A soil thermometer exposed at 2 feet deep in the same grove, 100 yards distant, furnished the soil temperature comparison. Observations were begun at 3 p. m. on February 18, 1918, a heavy snowstorm in the mountains surrounding the Coachella Valley indicating that a sharp frost might be expected on the following morning. Somewhat irregular observations were kept upon these thermometers during the next three months, from early morning till 9 or 10 p. m., in a few instances until a later hour, and one all-night record was made, the readings being taken from a half hour to two hours apart, according to the critical period of the record. Readings of the lower thermometer in the Zaheedy tree were discontinued for a time after March 15, and a second tree, a Maktoom,⁶ was used as a check, to ascertain whether the daily curve developed in the temperatures of the Zaheedy tree might be considered characteristic of the species.

These observations, continued until the end of May, became too voluminous for complete presentation within the scope of this article. Five periods of six to eight consecutive days each have been selected as bringing out most effectively the important points disclosed by these studies, and their records tabulated and in part presented in graphs in their relation to current air and soil temperatures.

The readings of the thermometers in the Zaheedy tree from February 18 to 25, inclusive, showing the response to the lowest temperatures encountered, are shown in Table I and the corresponding graphs in Figure 5. Table II shows the records of this tree from March 1 to 6, inclusive, a period when the daily air maxima were 80° F., but with the minima of the last two days suddenly lifted from between 46° and 48° to above 56°.

⁶ For convenience referred to as the Zaheedy and Maktoom trees.

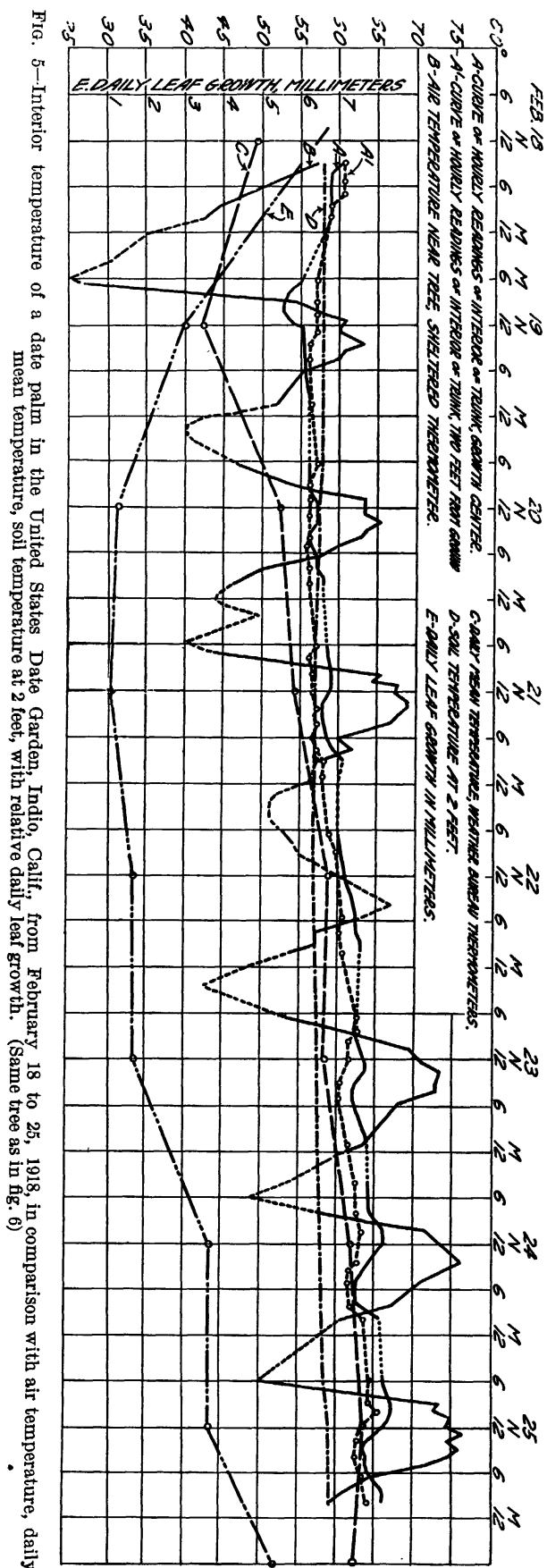


FIG. 5.—Interior temperature of a date palm in the United States Date Garden, Indio, Calif., from February 18 to 25, 1918, in comparison with air temperature, daily mean temperature, soil temperature at 2 feet, with relative daily leaf growth. (Same tree as in fig. 6)

TABLE I.—Interior temperatures of the date palm compared with air and soil temperatures of corresponding hours, February 18 to 25

Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)	Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)
Feb. 18:	°F.	°F.	°F.	°F.	Feb. 22:	°F.	°F.	°F.	°F.
3.00 p. m.---	57.20	-----	60.80	60.80	6.30 a. m.---	57.70	-----	60.35	59.00
4.00 p. m.---	55.40	-----	59.00	60.80	8.00 a. m.---	54.50	-----	60.35	59.90
7.00 p. m.---	48.20	-----	59.00	60.80	5.30 p. m.---	63.50	57.0	62.60	60.80
8.35 p. m.---	44.60	58.0	59.00	59.00	7.30 p. m.---	57.20	-----	62.60	60.35
10.00 p. m.---	42.80	-----	59.00	59.00	9.00 p. m.---	57.35	-----	<i>63.05</i>	60.80
Feb. 19:					10.00 p. m.---	53.60	-----	63.05	60.80
6.25 a. m.---	a 26.60	-----	55.40	57.20	Feb. 23:				
9.00 a. m.---	54.50	-----	52.70	57.20	6.30 a. m.---	<i>53.60</i>	-----	62.60	<i>62.60</i>
10.30 a. m.---	57.56	-----	52.70	57.20	8.15 a. m.---	60.80	-----	62.15	62.60
11.30 a. m.---	60.80	-----	53.60	57.20	9.30 a. m.---	65.35	-----	62.60	61.70
12.30 p. m.---	59.90	58.0	55.40	57.20	10.30 a. m.---	69.35	57.5	63.05	61.70
1.30 p. m.---	59.90	-----	55.40	57.20	12.00 a. m.---	70.70	-----	63.50	61.60
2.30 p. m.---	<i>b 63.50</i>	-----	55.40	56.30	1.30 p. m.---	73.40	-----	63.95	60.80
3.30 p. m.---	60.80	-----	55.40	56.30	3.00 p. m.---	72.95	-----	62.60	60.80
4.30 p. m.---	59.80	-----	55.40	56.30	4.00 p. m.---	72.95	-----	62.15	60.35
6.00 p. m.---	55.40	-----	55.40	56.30	5.30 p. m.---	68.00	-----	62.15	60.35
10.30 p. m.---	51.80	-----	56.30	56.30	11.00 p. m.---	63.50	-----	64.40	61.70
Feb. 20:					Feb. 24:				
6.20 a. m.---	46.40	-----	56.30	<i>57.20</i>	4.00 a. m.---	53.60	-----	64.40	62.60
9.00 a. m.---	53.60	-----	56.30	56.30	8.00 a. m.---	58.10	-----	64.40	62.60
10.00 a. m.---	58.10	-----	56.30	56.30	10.20 a. m.---	71.60	-----	<i>66.20</i>	<i>63.50</i>
11.00 a. m.---	63.50	-----	<i>57.20</i>	56.30	12.30 p. m.---	72.95	-----	66.20	62.60
12.00 a. m.---	63.50	-----	57.20	56.30	2.30 p. m.---	<i>76.10</i>	58.0	64.40	62.60
1.00 p. m.---	63.50	-----	57.20	56.30	3.30 p. m.---	74.30	-----	63.50	61.70
2.00 p. m.---	<i>65.30</i>	57.5	57.20	56.30	5.00 p. m.---	70.88	-----	62.60	61.70
3.10 p. m.---	63.50	-----	56.30	56.30	8.00 p. m.---	67.10	-----	62.60	61.70
4.00 p. m.---	62.60	-----	56.30	56.30	9.00 p. m.---	63.95	-----	64.40	62.60
5.00 p. m.---	59.90	-----	56.30	55.94	10.00 p. m.---	60.35	-----	65.75	63.50
6.30 p. m.---	54.50	-----	57.20	56.30	Feb. 25:				
8.00 p. m.---	50.00	-----	57.20	56.30	5.40 a. m.---	52.70	-----	66.30	64.40
9.00 p. m.---	48.20	-----	58.10	56.30	6.25 a. m.---	52.25	-----	66.20	64.40
10.00 p. m.---	46.40	-----	58.10	56.30	9.00 a. m.---	72.95	-----	67.10	64.40
Feb. 21:					10.00 a. m.---	72.50	-----	67.10	<i>65.30</i>
6.15 a. m.---	43.70	-----	58.55	<i>57.20</i>	11.00 a. m.---	74.75	-----	67.10	64.40
7.00 a. m.---	43.70	-----	58.55	56.30	12.00 a. m.---	74.30	59.0	66.20	63.95
9.45 a. m.---	65.30	-----	<i>69.00</i>	56.75	1.00 p. m.---	<i>76.55</i>	-----	65.30	63.50
10.30 a. m.---	67.40	-----	59.00	56.75	2.00 p. m.---	74.30	-----	63.95	63.05
11.00 a. m.---	67.55	-----	59.00	56.75	3.00 p. m.---	76.10	-----	63.50	62.60
12.00 a. m.---	67.10	-----	59.00	56.75	4.00 p. m.---	74.48	-----	63.95	62.60
1.00 p. m.---	<i>68.90</i>	-----	58.10	<i>56.75</i>	5.00 p. m.---	71.60	-----	63.95	62.60
2.00 p. m.---	68.90	-----	58.10	57.20	6.30 p. m.---	64.40	-----	64.40	63.50
3.00 p. m.---	67.55	57.0	58.10	56.75	8.30 p. m.---	60.80	-----	66.20	63.50
4.00 p. m.---	66.65	-----	58.10	57.20	10.00 p. m.---	59.00	-----	66.20	64.40
6.00 p. m.---	59.45	-----	58.55	56.75					
7.30 p. m.---	61.70	-----	59.00	57.20					
9.00 p. m.---	57.20	-----	60.80	58.10					
10.00 p. m.---	57.20	-----	60.35	58.10					

^a Figures in bold-face indicate lowest temperatures for the day.

^b Figures in italic indicate highest temperatures for the day.

TABLE II.—Interior temperatures of the date palm compared with air and soil temperatures of corresponding hours, March 1 to 6

Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)	Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)
Mar. 1:	°F.	°F.	°F.	°F.	Mar 4—Contd.	°F.	°F.	°F.	°F.
6.20 a. m.---	^a 46.40	-----	62.60	^b 63.05	6.30 p. m.---	67.55	-----	68.00	65.30
8.00 a. m.---	63.95	-----	63.14	63.05	7.30 p. m.---	66.65	-----	68.90	65.30
10.00 a. m.---	71.60	-----	<i>64.40</i>	63.05	9.00 p. m.---	64.85	-----	68.90	66.20
12.00 a. m.---	73.85	-----	62.60	62.60	10.00 p. m.---	63.50	-----	68.90	66.20
1.00 p. m.---	<i>78.35</i>	60.5	62.60	62.15	Mar. 5:				
3.30 p. m.---	76.55	-----	62.15	62.15	6.15 a. m.---	56.75	-----	70.70	67.10
4.30 p. m.---	74.30	-----	62.60	62.15	7.00 a. m.---	59.45	-----	71.60	67.10
7.00 p. m.---	58.10	-----	63.95	60.80	7.45 a. m.---	68.90	-----	72.50	<i>68.00</i>
Mar. 2:					8.20 a. m.---	72.05	-----	72.50	68.00
10.00 a. m.---	74.30	-----	<i>65.30</i>	63.50	9.00 a. m.---	72.50	-----	<i>72.95</i>	67.55
12.00 a. m.---	78.80	-----	63.50	62.60	10.00 a. m.---	74.30	-----	72.95	67.28
2.00 p. m.---	<i>79.70</i>	-----	63.50	62.15	10.30 a. m.---	77.45	-----	72.50	67.10
3.30 p. m.---	78.80	-----	63.65	61.70	11.00 a. m.---	77.90	-----	71.15	67.28
7.00 p. m.---	55.40	61.5	64.40	63.05	12.00 a. m.---	79.25	-----	70.34	67.10
9.00 p. m.---	57.80	-----	65.75	63.95	1.00 p. m.---	77.90	62.5	69.08	66.74
10.00 p. m.---	54.50	-----	66.20	63.50	2.15 p. m.---	<i>79.70</i>	-----	68.54	66.20
Mar. 3:					3.00 p. m.---	76.55	-----	68.45	65.30
6.15 a. m.---	47.75	-----	67.10	<i>65.30</i>	4.00 p. m.---	74.75	-----	69.36	65.75
7.15 a. m.---	57.95	-----	<i>67.55</i>	65.30	5.15 p. m.---	71.60	-----	68.36	66.20
11.00 a. m.---	78.80	-----	67.55	64.40	6.30 p. m.---	67.55	-----	69.80	66.20
1.00 p. m.---	79.70	62.0	65.30	63.50	7.30 p. m.---	65.30	-----	70.70	67.10
2.00 p. m.---	<i>79.88</i>	-----	63.50	63.14	9.00 p. m.---	65.30	-----	71.60	68.00
4.15 p. m.---	77.90	-----	63.50	63.05	Mar. 6:				
6.30 p. m.---	61.25	-----	66.20	63.50	6.10 a. m.---	54.95	-----	71.78	<i>69.80</i>
7.30 p. m.---	56.75	-----	66.20	64.40	7.45 a. m.---	61.25	-----	71.37	68.90
9.00 p. m.---	67.55	-----	67.55	64.40	8.30 a. m.---	62.78	-----	72.50	68.90
Mar. 4:					9.40 a. m.---	67.55	-----	72.50	68.90
6.10 a. m.---	45.95	-----	71.60	<i>67.10</i>	10.30 a. m.---	66.65	-----	<i>73.53</i>	68.90
7.45 a. m.---	58.55	-----	70.70	67.10	11.00 a. m.---	69.80	-----	73.40	68.45
8.45 a. m.---	61.70	-----	72.05	67.10	12.00 a. m.---	69.98	-----	72.95	68.00
10.00 a. m.---	66.65	-----	<i>72.50</i>	67.10	1.00 p. m.---	69.44	-----	72.05	67.55
11.00 a. m.---	71.15	-----	72.50	66.65	2.00 p. m.---	<i>70.70</i>	62.5	70.70	67.55
12.00 a. m.---	75.20	-----	71.15	66.20	3.00 p. m.---	69.35	-----	72.05	67.10
1.00 p. m.---	77.00	-----	69.35	65.75	4.00 p. m.---	67.55	-----	69.98	67.10
2.00 p. m.---	77.90	62.5	68.00	65.30	5.00 p. m.---	65.48	-----	69.80	67.10
3.00 p. m.---	78.08	-----	67.28	65.30	6.20 p. m.---	63.05	-----	69.80	67.10
4.00 p. m.---	77.90	-----	67.10	65.30	8.00 p. m.---	60.80	-----	71.60	67.10
5.00 p. m.---	72.05	-----	67.10	65.30	9.00 p. m.---	59.90	-----	71.60	67.10

^a Figures in bold-face indicate lowest temperatures for the day.
^b Figures in italic indicate highest temperatures for the day.

Table III shows similar records from March 26 to April 1, inclusive, during which time the minimum air temperatures passed above 60° while the maxima for March 30 and 31 were above 95°. Table IV and Figure 6 show the records of both upper and lower thermometers in the Zaheedy tree for the period from April 21 to 27, inclusive, where the holding down of the temperature of the lower trunk interior is most pronounced.

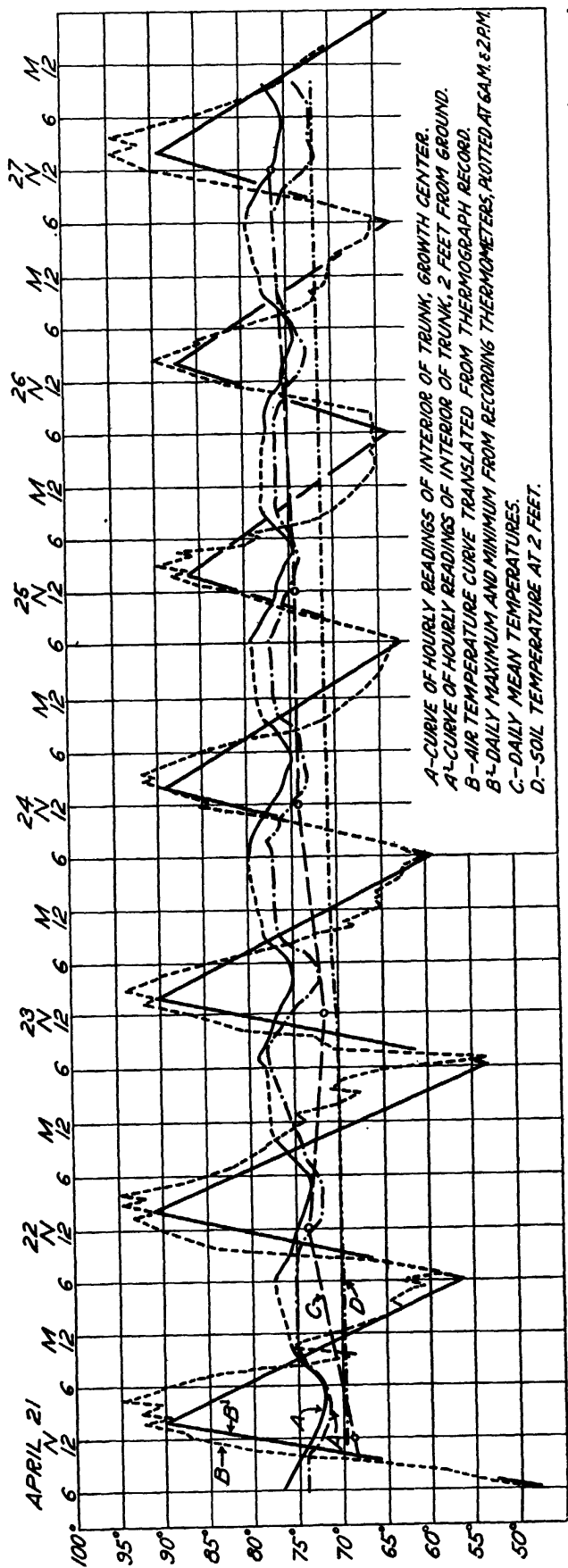


FIG 6—Hourly interior temperatures of a date palm in the United States Date Garden, Indio, Calif., from April 21 to 27, 1918, in comparison with air temperature from thermograph records, daily minimum, maximum, and mean temperature and soil temperature at 2 feet. (Same tree as in fig. 5)

TABLE III.—Interior temperatures of the date palm compared with air and soil temperatures of corresponding hours, March 26 to April 1

Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)	Time	Air	Soil at 2 feet	Bud zone (by thermometer 1)	Trunk (by thermometer 2)
Mar. 26:	°F.	°F.	°F.	°F.	Mar. 29—Contd.	°F.	°F.	°F.	°F.
5.30 a. m.	66.20		75.20	73.94	12.10 p. m.	86.90		71.60	68.90
6.30 a. m.	66.20		76.10	72.50	1.05 p. m.	89.60		71.60	68.90
9.30 a. m.	72.50		76.28	73.22	2.15 p. m.	<i>90.60</i>	66.0	71.60	68.90
10.30 a. m.	75.20		76.10	70.70	3.25 p. m.	88.70		70.34	69.80
11.30 a. m.	80.15		75.38	69.98	4.10 p. m.	87.80		70.70	69.80
1.00 p. m.	83.78		75.38	68.90	5.00 p. m.	86.45		70.70	69.80
2.40 p. m.	83.30		73.22	68.54	7.00 p. m.	75.65		71.60	72.05
3.45 p. m.	83.30	65.0	72.50	68.54	8.00 p. m.	70.25		72.50	72.05
4.30 p. m.	82.40		72.32	68.36	9.00 p. m.	69.80		73.40	72.50
6.30 p. m.	71.60		72.50	69.80	Mar. 30:				
7.45 p. m.	70.88		72.50	70.70	5.45 a. m.	71.78		76.10	73.40
9.15 p. m.	68.45		72.50	71.15	7.30 a. m.	79.60		77.46	72.95
10.00 p. m.	67.10		72.05	70.70	8.10 a. m.	84.65		76.55	72.50
Mar. 27:					9.00 a. m.	88.70		75.38	71.42
5.50 a. m.	62.60		70.70	71.60	10.00 a. m.	89.60		74.48	69.35
6.30 a. m.	63.95		70.70	72.14	11.00 a. m.	92.30		73.94	
7.17 a. m.	66.65		72.32	71.60	12.00 a. m.	<i>92.48</i>		72.95	68.90
8.00 a. m.	69.35		<i>74.30</i>	71.60	1.00 p. m.	92.48	66.5	72.05	68.90
8.30 a. m.	70.70		74.30	70.70	2.10 p. m.	92.30		71.15	68.90
9.30 a. m.	72.95		73.04	70.25	3.15 p. m.	92.30		70.34	68.90
10.45 a. m.	77.00		72.68	69.08	3.50 p. m.	89.60		70.52	69.08
11.50 a. m.	78.35		72.50	68.54	4.30 p. m.	90.32		70.34	69.08
1.00 p. m.	<i>80.15</i>	65.5	72.05	68.90	5.30 p. m.	85.10		71.06	70.25
2.00 p. m.	79.70		71.60	67.82	8.15 p. m.	71.60		72.95	73.40
2.30 p. m.	79.52		70.70	67.82	9.00 p. m.	71.15		73.40	73.40
3.00 p. m.	78.35		69.98	67.82	Mar. 31:				
3.50 p. m.	78.35		69.44	68.54	5.00 a. m.	64.40		76.55	<i>76.55</i>
4.30 p. m.	76.55		68.00	68.18	6.30 a. m.	67.55		77.00	75.20
6.10 p. m.	70.70		69.80	69.35	8.00 a. m.	79.25		77.00	73.94
7.30 p. m.	68.45		71.42	70.70	8.30 a. m.	82.85		76.55	73.40
8.30 p. m.	65.75		71.78	70.70	10.00 a. m.	92.30		75.20	71.60
9.30 p. m.	63.95		72.32	71.60	11.00 a. m.	93.20		74.30	70.70
Mar. 28:					12.10 p. m.	93.65		72.95	69.80
6.00 a. m.	52.70		73.22	<i>72.32</i>	1.00 p. m.	94.55		72.50	69.62
7.00 a. m.	68.45		<i>75.02</i>	71.42	2.50 p. m.	94.55	67.0	70.70	69.35
8.10 a. m.	72.50		74.12	70.70	4.00 p. m.	<i>95.90</i>		69.98	69.35
9.15 a. m.	77.00		73.40	69.80	6.30 p. m.	80.15		69.98	69.80
10.10 a. m.	83.75		72.50	69.80	7.30 p. m.	76.55		71.15	72.50
11.00 a. m.	84.20		71.60	69.35	8.30 p. m.	74.75		69.80	73.40
12.05 p. m.	82.85		71.60	68.90	9.30 p. m.	73.85		73.85	73.40
1.10 p. m.	84.65	65.5	71.60	68.90	Apr. 1:				
2.20 p. m.	<i>86.90</i>		68.90	68.45	6.30 a. m.	63.68		78.35	<i>76.55</i>
3.10 p. m.	86.00		70.70	69.80	7.30 a. m.	65.30		77.90	76.10
4.00 p. m.	85.10		70.70	69.35	8.15 a. m.	68.36		77.18	75.20
5.00 p. m.	81.05		69.80	68.90	9.30 a. m.	71.60		76.10	74.30
7.00 p. m.	69.80		71.15	71.60	10.30 a. m.	75.65		75.20	73.85
8.10 p. m.	64.40		71.78	71.60	11.00 a. m.	77.45		74.75	73.40
9.00 p. m.	64.94		72.50	73.40	12.00 a. m.	78.35		73.85	73.94
10.15 p. m.	71.60		74.30	<i>74.30</i>	1.15 p. m.	77.00		72.95	70.70
Mar. 29:					3.00 p. m.	77.00		72.32	70.70
5.30 a. m.	56.30		73.40	72.14	3.30 p. m.	77.00		72.14	70.70
7.20 a. m.	76.10		75.38	71.60	4.00 p. m.	75.65		71.60	70.70
8.20 a. m.	78.44		75.20	71.42	5.00 p. m.	72.95		71.42	71.24
9.24 a. m.	82.40		73.40	69.80	7.00 p. m.	65.75		71.78	71.78
10.05 a. m.	83.75		72.50	69.80	8.00 p. m.	63.95		72.95	71.60
11.15 a. m.	85.10		71.96	69.35	9.00 p. m.	63.50		73.40	72.50

° Figures in bold-face type indicate lowest temperature for the day.

° Figures in italic indicate highest temperature for the day.

TABLE IV.—Interior temperatures of the date palm compared with air and soil temperatures of corresponding hours, April 21 to April 27

Time	Air	Soil at 2 feet	Zaheedy (by thermometer 1)	Zaheedy (by thermometer 2)	Relative humidity	Time	Air	Soil at 2 feet	Zaheedy (by thermometer 1)	Zaheedy (by thermometer 2)	Relative humidity	
Apr. 21:	°F.	°F.	°F.	°F.	P. ct.	Apr. 24—Con.	°F.	°F.	°F.	°F.	P. ct.	
6.30 a. m.	64	-----	77.00	74.00	27	4.45 p. m.	90	-----	74.60	73.00	28	
10.00 a. m.	75	70.00	74.50	74.00	-----	5.45 p. m.	86	-----	74.98	73.75	-----	
11.00 a. m.	82	-----	74.00	73.00	-----	6.15 p. m.	84	-----	74.90	73.50	-----	
12.15 p. m.	87	-----	73.50	72.00	-----	7.35 p. m.	77	-----	75.20	73.50	-----	
2.40 p. m.	92	69.90	72.50	71.50	-----	8.30 p. m.	74	-----	76.00	-----	-----	
5.30 p. m.	92	-----	72.00	72.00	23	9.30 p. m.	71	70.75	77.60	76.00	-----	
6.45 p. m.	88	-----	72.50	72.50	-----	Apr. 25:	6.30 a. m.	63	71.00	79.00	77.00	-----
8.20 p. m.	74	-----	74.50	74.75	-----	7.40 a. m.	67	-----	78.10	76.30	-----	
9.00 p. m.	70	-----	75.00	75.00	-----	8.55 a. m.	72	-----	77.50	75.45	18	
10.00 p. m.	75	-----	75.00	75.00	-----	9.45 a. m.	74	-----	77.00	75.50	-----	
Apr. 22:	-----	-----	-----	-----	-----	10.45 a. m.	78	-----	76.50	75.50	-----	
6.20 a. m.	58	69.90	77.25	75.00	-----	11.45 a. m.	79	-----	76.25	75.00	-----	
9.00 a. m.	82	-----	75.50	74.75	-----	12.30 p. m.	82	71.00	76.00	74.50	-----	
10.15 a. m.	86	-----	75.00	74.50	23	1.30 p. m.	87	-----	75.60	74.00	-----	
11.00 a. m.	88	-----	75.00	73.50	-----	2.45 p. m.	90	-----	75.00	74.00	-----	
12.00 a. m.	90	70.00	74.50	72.50	-----	3.45 p. m.	87	-----	74.50	73.50	-----	
1.30 p. m.	93	-----	74.25	72.25	-----	4.30 p. m.	85	-----	74.00	74.00	37	
3.00 p. m.	95	-----	73.50	72.00	-----	6.30 p. m.	78	71.00	75.25	74.50	-----	
4.00 p. m.	95	-----	73.00	72.00	-----	7.30 p. m.	74	-----	76.00	75.00	-----	
5.00 p. m.	90	70.00	73.00	72.00	18	8.30 p. m.	71	-----	77.00	75.80	-----	
7.15 p. m.	84	-----	74.50	73.75	-----	9.30 p. m.	70	-----	77.50	76.00	-----	
8.10 p. m.	79	-----	75.50	74.00	-----	Apr. 26:	6.30 a. m.	64	71.00	77.00	76.00	-----
9.15 p. m.	78	-----	76.50	74.00	-----	8.00 a. m.	66	-----	77.00	76.00	-----	
10.00 p. m.	77	-----	77.00	74.50	-----	9.00 a. m.	70	-----	77.00	75.60	56	
Apr. 23:	-----	-----	-----	-----	-----	10.00 a. m.	73	-----	76.50	75.25	-----	
6.15 a. m.	53	70.00	78.00	77.50	-----	11.30 a. m.	81	-----	75.50	74.75	-----	
7.05 a. m.	57	-----	79.00	77.50	57	1.30 p. m.	85	-----	75.00	72.95	-----	
8.15 a. m.	72	-----	77.80	77.98	-----	3.00 p. m.	90	-----	74.50	72.50	-----	
9.30 a. m.	74	-----	77.50	77.10	-----	4.10 p. m.	87	-----	74.00	72.95	-----	
10.45 a. m.	83	-----	77.00	76.30	-----	5.00 p. m.	85	-----	73.75	73.40	32	
11.25 a. m.	85	-----	76.90	76.00	-----	7.00 p. m.	75	71.25	74.50	74.30	-----	
12.30 p. m.	87	70.00	76.50	75.20	-----	8.30 p. m.	72	-----	76.00	-----	-----	
1.45 p. m.	92	-----	75.50	73.40	-----	9.30 p. m.	72	-----	77.00	75.20	-----	
2.30 p. m.	94	-----	75.00	72.20	-----	10.30 p. m.	71	-----	77.10	75.20	-----	
3.40 p. m.	92	-----	75.00	72.10	23	Apr. 27:	6.30 a. m.	65	71.25	79.00	76.10	-----
4.20 p. m.	90	-----	75.00	72.10	-----	8.10 a. m.	70	-----	78.40	75.38	57	
5.30 p. m.	87	-----	75.00	72.30	-----	9.40 a. m.	78	71.40	77.80	74.75	-----	
6.30 p. m.	82	70.50	75.75	73.00	-----	10.30 a. m.	82	-----	76.75	74.30	-----	
7.30 p. m.	77	-----	76.50	75.10	-----	11.30 a. m.	84	-----	75.90	72.50	-----	
8.30 p. m.	73	-----	77.30	76.00	-----	12.35 p. m.	86	71.50	75.95	71.60	-----	
9.30 p. m.	71	-----	78.00	76.50	-----	1.45 p. m.	90	-----	75.80	71.42	-----	
Apr. 24:	-----	-----	-----	-----	-----	2.35 p. m.	92	-----	75.80	71.60	-----	
6.30 a. m.	61	70.50	79.50	77.00	-----	3.40 p. m.	93	71.50	75.00	71.60	-----	
7.30 a. m.	65	-----	79.00	77.50	59	4.30 p. m.	92	-----	74.95	71.68	-----	
8.10 a. m.	68	-----	78.90	76.90	-----	5.30 p. m.	90	-----	74.95	71.60	20	
9.30 a. m.	73	-----	78.40	76.30	-----	6.30 p. m.	86	-----	74.95	71.60	-----	
10.25 a. m.	77	-----	78.10	75.90	-----	8.30 p. m.	78	71.00	76.00	72.50	-----	
11.30 a. m.	80	-----	77.20	74.40	-----	9.30 p. m.	74	-----	76.75	73.40	-----	
12.45 p. m.	85	-----	76.10	73.80	-----							
1.30 p. m.	88	-----	76.00	73.20	-----							
2.30 p. m.	92	-----	75.50	73.00	-----							
3.20 p. m.	91	-----	75.00	73.00	-----							

^a Figures in bold-face type indicate lowest temperature for the day.

^b Figures in italic indicate highest temperature for the day.

Table V shows the records of the Maktoom tree at the growth center and of the Zaheedy tree at 2 feet from the ground, from April 28 to May 4, a period when the interior temperature curves both fell mainly below the curve of the mean daily air temperature. Table VI shows temperatures observed in May, 1923, in the trunk of a large seedling male palm at 4 feet from the ground, compared with soil temperatures at 2 feet and 3 feet deep. Here, with an older and more deeply rooted tree, a striking proof of the influence of the ascending sap current is introduced, in that the tree trunk temperature passed below that of the soil at 2 feet for several days, and closely approached the soil temperature given by the thermometer set at a depth of 3 feet.

TABLE V.—Interior temperatures of the date palm compared with air and soil temperatures of corresponding hours, April 28 to May 4

Time	Air	Soil	Mak-toom (by ther- mometer 1)	Za-heedy (by ther- mometer 2)	Mean air	Time	Air	Soil	Mak-toom (by ther- mometer 1)	Za-heedy (by ther- mometer 2)	Mean air
Apr. 28:	°F.	°F.	°F.	°F.	°F.	May 1—Con.	°F.	°F.	°F.	°F.	°F.
6.30 a. m.	^a 64	70.50	^b 75.20	76.10	-----	7.45 p. m.	85	70.50	77.36	73.76	-----
7.15 a. m.	67	-----	75.20	76.10	-----	8.30 p. m.	77	-----	78.98	76.10	-----
9.30 a. m.	77	-----	75.20	75.20	-----	9.30 p. m.	70	-----	79.70	76.64	-----
10.40 a. m.	86	71.00	74.75	74.48	-----	May 2:					
11.45 a. m.	89	-----	74.30	74.30	-----	6.00 a. m.	55	70.00	80.60	77.45	-----
1.30 p. m.	91	-----	73.40	73.22	76.7	7.30 a. m.	62	-----	79.70	77.00	-----
2.45 p. m.	<i>94</i>	-----	73.85	73.40	-----	8.45 a. m.	74	-----	78.80	77.00	-----
3.45 p. m.	<i>94</i>	71.00	72.50	73.58	-----	10.00 a. m.	78	-----	77.90	76.82	-----
4.45 p. m.	91	-----	73.85	73.40	-----	11.00 a. m.	83	-----	77.90	76.64	-----
6.30 p. m.	85	-----	74.30	74.30	-----	12.30 p. m.	90	-----	77.00	75.74	-----
7.00 p. m.	82	-----	74.50	74.75	-----	2.30 p. m.	97	-----	77.00	74.75	78.0
8.30 p. m.	78	-----	75.20	75.20	-----	3.30 p. m.	99	-----	76.10	74.30	-----
9.30 p. m.	76	-----	75.20	75.20	-----	4.30 p. m.	<i>101</i>	70.50	76.10	74.30	-----
Apr. 29:						5.30 p. m.	95	-----	76.10	73.40	-----
6.00 a. m.	69	-----	77.00	77.00	-----	6.30 p. m.	90	-----	76.10	73.58	-----
6.40 a. m.	64	70.75	77.00	76.82	-----	7.30 p. m.	82	-----	76.55	73.58	-----
8.50 a. m.	80	-----	76.10	75.74	-----	8.30 p. m.	73	70.50	77.00	75.56	-----
10.10 a. m.	86	-----	75.65	75.20	-----	May 3:					
11.50 a. m.	88	-----	74.30	75.20	-----	6.00 a. m.	60	71.00	<i>79.25</i>	79.70	-----
2.25 p. m.	95	-----	73.85	71.60	78.5	7.10 a. m.	61	-----	79.16	79.25	-----
3.05 p. m.	<i>96</i>	-----	73.40	71.60	-----	8.30 a. m.	73	-----	78.80	78.35	-----
4.03 p. m.	95	71.00	73.85	71.78	-----	9.35 a. m.	80	-----	78.35	77.90	-----
5.00 p. m.	94	-----	73.85	72.95	-----	10.30 a. m.	85	-----	77.90	75.65	-----
6.20 p. m.	90	-----	74.30	73.40	-----	11.30 a. m.	90	-----	77.36	75.38	80.0
7.07 p. m.	85	-----	74.75	75.20	-----	12.30 p. m.	92	-----	77.00	75.20	-----
8.30 p. m.	81	-----	75.65	-----	-----	1.40 p. m.	95	-----	75.65	75.20	-----
9.30 p. m.	74	-----	76.10	76.10	-----	2.45 p. m.	<i>99</i>	-----	75.56	74.30	-----
Apr. 30:						3.45 p. m.	98	-----	75.30	73.76	-----
6.12 a. m.	60	70.00	77.00	77.90	-----	4.30 p. m.	97	-----	75.20	73.76	-----
7.25 a. m.	70	-----	77.18	77.45	-----	5.30 p. m.	95	-----	75.20	73.76	-----
9.50 a. m.	83	-----	77.18	76.55	-----	6.30 p. m.	90	71.00	75.20	74.30	-----
10.20 a. m.	85	-----	76.55	75.60	-----	7.30 p. m.	85	-----	76.10	-----	-----
11.40 a. m.	92	-----	76.10	75.38	-----	May 4:					
1.15 p. m.	<i>96</i>	-----	75.20	74.75	-----	6.15 a. m.	60	70.60	80.15	79.70	-----
2.15 p. m.	96	-----	74.30	72.50	-----	7.30 a. m.	65	-----	80.15	78.80	-----
4.25 p. m.	91	-----	75.20	73.40	79.0	8.30 a. m.	75	-----	79.70	78.35	-----
6.52 p. m.	85	-----	76.10	75.20	-----	9.30 a. m.	79	-----	78.80	78.08	-----
8.00 p. m.	80	70.50	77.00	76.10	-----	10.30 a. m.	83	-----	77.90	78.08	-----
9.30 p. m.	76	-----	78.35	77.00	-----	11.30 a. m.	85	-----	77.00	77.90	-----
May 1:						12.35 p. m.	87	71.50	76.64	76.64	-----
6.30 a. m.	70	70.00	80.60	78.80	-----	1.30 p. m.	90	-----	76.55	75.56	-----
7.30 a. m.	73	-----	78.35	77.54	-----	2.25 p. m.	92	-----	76.55	74.93	77.0
8.30 a. m.	79	-----	77.72	77.00	-----	3.30 p. m.	<i>93</i>	-----	76.10	74.75	-----
9.30 a. m.	85	-----	77.45	76.28	-----	4.30 p. m.	92	-----	76.10	74.75	-----
11.13 a. m.	88	-----	77.00	-----	-----	5.30 p. m.	91	-----	76.10	74.75	-----
1.10 p. m.	93	70.50	75.65	74.75	-----	6.30 p. m.	87	-----	76.55	75.02	-----
3.30 p. m.	<i>97</i>	-----	73.40	72.50	-----	7.30 p. m.	80	-----	77.36	76.10	-----
5.10 p. m.	96	-----	75.20	72.95	-----	8.30 p. m.	78	71.50	78.44	76.82	-----
6.30 p. m.	93	-----	76.64	73.40	82.5						

^a Figures in bold-face type indicate lowest temperature for the day
^b Figures in italic indicate highest temperature for the day

TABLE VI.—Interior temperatures of a large seedling male palm compared with air and soil temperatures, May 5 to May 11, 1923

Time	Air (by thermo- graph)	Soil at 2 feet	Soil at 3 feet	Interior of trunk (at 4 feet height)	Time	Air (by thermo- graph)	Soil at 2 feet	Soil at 3 feet	Interior of trunk (at 4 feet height)
May 5:	° F.	° F.	° F.	° F.	May 9—Contd.	° F.	° F.	° F.	° F.
8.30 a. m.---	89	72. 75	-----	73. 00	10.45 a. m.---	-----	73. 75	71. 55	73. 75
10. 00 a. m.---	90	73. 00	-----	72. 00	12.00 a. m.---	103	73. 75	71. 55	73. 50
12.00 a. m.---	-----	73. 00	-----	71. 75	1.30 p. m.---	-----	73. 75	71. 55	73. 25
2.00 p. m.---	<i>a</i> 102	73. 00	-----	71. 75	3.00 p. m.---	-----	73. 75	71. 55	73. 50
4.00 p. m.---	-----	73. 00	-----	<i>b</i> 71. 50	4.00 p. m.---	-----	73. 75	71. 55	73. 25
May 7:	-----	-----	-----	-----	5.00 p. m.---	105	73. 75	71. 55	73. 50
8.15 a. m.---	85	74. 00	-----	74. 50	May 10:	-----	-----	-----	-----
11.00 a. m.---	-----	74. 00	-----	73. 00	8.30 a. m.---	87	74. 00	72. 00	75. 75
1.30 p. m.---	-----	<i>74. 50</i>	72. 00	<i>b</i> 72. 50	10.00 a. m.---	-----	74. 00	71. 55	74. 00
3.00 p. m.---	110	74. 00	71. 10	72. 50	11.00 a. m.---	-----	<i>74. 25</i>	71. 55	73. 75
4.45 p. m.---	-----	74. 25	71. 10	72. 50	12.00 a. m.---	100	74. 25	71. 55	73. 75
May 8:	-----	-----	-----	-----	1.30 p. m.---	-----	74. 25	71. 55	73. 50
8.00 a. m.---	90	75. 00	71. 10	75. 00	3.30 p. m.---	103	74. 25	71. 55	73. 50
10.00 a. m.---	-----	74. 75	71. 10	73. 00	5.00 p. m.---	-----	74. 00	71. 55	73. 50
11.00 a. m.---	-----	75. 00	71. 10	72. 75	May 11:	-----	-----	-----	-----
1.30 p. m.---	109	75. 00	71. 10	72. 75	9.00 a. m.---	80	74. 50	72. 00	75. 50
2.30 p. m.---	-----	75. 00	71. 10	72. 50	10.30 a. m.---	-----	74. 50	72. 00	74. 25
4.00 p. m.---	-----	75. 00	70. 65	72. 75	11.30 a. m.---	95	74. 50	72. 00	74. 00
May 9:	-----	-----	-----	-----	1.45 p. m.---	102	74. 50	72. 00	73. 75
8.15 a. m.---	86	<i>74. 50</i>	72. 00	<i>75. 50</i>	3.15 p. m.---	-----	<i>74. 75</i>	72. 00	73. 50
9.45 a. m.---	-----	74. 00	72. 00	74. 00	4.30 p. m.---	95	74. 50	72. 00	74. 00

a Figures in bold-face type indicate lowest temperature for the day.

b Figures in italic indicate highest temperature for the day.

THE INVERTED TEMPERATURE CURVE

Tables I to VI and the graphs shown in Figures 5 and 6 indicate a fairly uniform and very significant 24-hour curve of the interior tree temperature. In February and early March a daily maximum was reached at about 9 to 10 or 11 a. m., but as the air temperature means advanced during the latter part of March, this interior maximum occurred about 6.30 a. m., and was maintained but a short time, followed by more or less gradual drop of from 2° or 3° to 5°, or at most 7°, reaching a minimum at from 3 to 5 p. m. From this a rise followed, unless the air temperature had dropped during the day, persisting as late in the night as the readings were taken, which was usually not later than 10:30 p. m., although one record was kept to 12.30 a. m., and one all night. A continuation of this rise was generally traced at 4.30 to 6.30 a. m., making the night temperature curve a part of the approach to the morning maximum.

Whatever the factors may be, tending toward an equalization of the temperature of the growing tissues, the result is an inversion of the temperatures, holding the tissues at their highest point during the coolest morning hours, and at their lowest temperature during the maximum of the day's heat.

NARROW RANGE OF INTERIOR TEMPERATURES

Another matter of interest to be noted in Tables I to VI and in the graphs Figures 5 and 6, is the very narrow daily range exhibited by the interior temperatures as compared with the temperature of the air. During February and the early part of March the 24-hour range shown by the upper thermometer inserted into the growing region of the date trunk was only from 1.8° to 5.4°, and that of the

lower thermometer only from 0.36° to 2.7° , while for the corresponding period the air temperatures range from 21° to 38° . During the last days of March, with maximum air temperatures reaching 94° and 95° , and minima at 60° and 61° , a daily range of 33° to 35° , the 24-hour range of temperatures within the two trees was at most 7.2° .

Next we may note that the tree temperatures were slowly ascending, day by day, at a rate which on the whole corresponded with the rise in the daily mean temperature of the air, tending at first to keep above it, but as the mean air temperature increased, falling below it, but throughout keeping a relatively uniform distance above the soil temperature.

Here it is necessary to consider more in detail the action of the factors (1) and (3), previously outlined, in their control of the temperature of the growth center.

THE AIR TEMPERATURE

Thermographic records show that at the Indio garden, the air minimum is usually reached shortly before sunrise and is maintained but a short time. The upward curve of the trace is a rapid one, often showing a gain of 20° by 9 or 10 a. m., with a maximum for the day 25° to 40° above the minimum; reached at 2 or 3, sometimes as late as 4 p. m. The afternoon decline is much slower than the morning rise, until after sunset, when the radiation through the dry desert air goes on steadily till morning.

The regulatory influence of the daily air temperature naturally falls into two periods—(1) in which the air temperature is below the soil temperature; and (2) that in which the air temperature is above the soil temperature. With the preponderance of the first, the growth center temperature, after the lag in penetrating the protection envelope, is pulled down toward the soil temperature and, if severe enough, is brought below it; the sap, deriving its temperature from the soil, being unable to wholly overcome the penetration of cold from without.

This condition is shown in Table I and Figure 5, where from 3 p. m. on February 18 to 7 a. m. on the 21st, the air temperature was below the soil temperature for 46 hours and above it only 18 hours. This is expressed by the mean temperature line *C* Figure 5, which remained below the soil temperature line until the 22d.

The penetration, through the protecting envelope, was slow, however, and the growth center minimum of 52.7° was only reached at from 9 to 10.30 a. m. The sap, at about the soil temperature, must be credited with overcoming this frost incursion and keeping the growth center temperature a little above the zero point of 50° .

The thermometers show both trunk and bud temperatures below the soil temperature, with a slow recovering on the afternoon of the 21st. The soil temperature, itself, under the influence of the prolonged low air temperature was lowered from 58° to 57.5° and finally to 57° , only getting back to 58° on the 24th after the air temperature curve became higher than the soil curve for a predominant portion of the day.

In successful date-producing regions the periods when the air is colder than the soil are very short and the main problem is with the

air warmer than the soil for a greater part of the day, or ultimately for the entire 24 hours, as is shown in Table VI. An inspection of Tables I to V shows that on a clear day the air is maintained at within about 5° or 6° of the day's maximum for a period of from 5 to 6 hours, and within 10° of the maximum for an average of 7 hours. Upon exposed surfaces the penetration of heat, when the maximum air temperature is 100° or upward, is terrific and the radiation of the accumulated heat from a brick or adobe wall can be felt long after sundown. Vegetation with unprotected surfaces often shows, within the tissues, temperatures several degrees above the surrounding air. Smith (23) observed interior temperatures in the giant bamboo, *Dendrocalamus giganteus*, 6° above the surrounding air, which he attributed to "the intensity of the solar radiation incident upon the growing organ." Even greater increment in temperature has been observed in the tissue of the giant cactus upon the deserts of the Southwest. The different conditions found in the interior of the date palm are in most striking contrast.

To sum up the evidence, then, the air temperatures, low or high, are retarded and largely intercepted by the protective layer of leaf bases and sheath fiber, before they reach the merismatic tissues of the growth center or phyllophore. This is a stabilizing effect, tending to prevent the penetration of temperatures below the optimum for cell growth in cold weather, and conversely, retarding the penetration of temperatures above the optimum during periods of extreme heat.

THE SOIL TEMPERATURE

The second factor, derived from the first, is the temperature of the soil at 2 to 3 feet deep, where the greater part of the young date palm roots are located. This determines the temperature of the soil moisture and must largely govern the temperature of the ascending sap current.

In Indio the soil temperature at 2 feet deep is surprisingly constant, the diurnal range during the period of the experiment not exceeding 0.5° , and during the heat of the summer not exceeding 1° to 1.5° F.

The relatively low temperature of the Indio soil can be attributed in part to its peculiar physical character. The deeper strata are composed of the old lake bed silts, above which are alternating layers of fine silts from the widely dispersed shallow flooding from the Whitewater River and wind-blown fine sand with a high proportion of fine particles of mica. It has a high water capacity without the danger of becoming "puddled" or impervious to air as does a clay soil with repeated wetting.

The ample irrigation practiced at this garden doubtless is largely responsible for the soil being retained at a moderate temperature. The mean air temperature for June, July, and August, 1918, was 91° . The temperature of the water from the artesian wells used in irrigation is about 74° . With an irrigation every 10 to 14 days, the cooling effect of this volume of water, with the added cooling of evaporation would result in keeping the soil temperature several degrees below the mean air temperature of the hottest weather. Free (7, p. 188) attaches much importance to the high specific heat of soil water. From his statements we may conclude that the retarded warming up of the soil due to water, a disadvantage in the spring on an eastern farm,

becomes a positive advantage under the extreme heat of a desert summer. The seasonal range of soil temperature at the Indio garden at the depth of 2 feet is a narrow one, extending in 1918 from 57° in January to 85.5° in August, an amplitude of only 28.5° . For comparison, soil temperature studies by Bouyoucos at the Michigan Agricultural Experiment Station in 1913 (1, p. 63, 68) may be quoted, showing in loam soil at 18 inches deep a minimum temperature of 31.3° in February, and a maximum of 76.9° in July, giving a yearly amplitude of 45.6° . The Indio date garden soil temperature at 2 feet declined from 58° on February 18 to 57° on February 21, then slowly advanced to 67° by March 31 and to 71.5° on May 4 (the time covered in Tables I to V).

INFLUENCE OF THE ASCENDING SAP CURRENTS

In the records from February 18 to 21, Table I, also from March 9 to 15, and again from March 19 to 21, the position of the daily air mean below the soil temperature, and the close correspondence of the tree temperature with that of the soil point unquestionably to the sap current as a controlling temperature factor.

Most significant is the record of the morning of February 19 (Table I and fig. 5), when there was a sharp frost, with an air temperature of 26.6° F. beside the tree (standard minimum in Weather Bureau shelter, a few hundred yards distant, 25°). At 6.30 a. m. the tree record was 55.4° , which slowly declined to 52.7° between 9 a. m. and 10.30 a. m., then ascended in the afternoon to 55.4° .

The thermograph trace kept near the tree shows that the air temperature was below the freezing point for 5 hours, from 2.15 to 6.45 a. m., so we must conclude that the freezing temperature produced its greatest depressing effect on the interior tissues of the bud only after a lag of from 7 to 8 hours. The lower thermometer in the trunk did not reach its minimum of 56.3° until 2.30 p. m., showing here, as do the subsequent records, that the trunk tissues are less sensitive to the air temperatures than those of the bud.

With the thermometer in the bud zone showing a drop to only 5.3° below the soil temperature of 58° , and the lower or trunk thermometer indicating only 2° below the soil temperature, it is evident that the ascending sap current must have been the controlling factor, protected from air temperature by the insulating outer layers. Although the movement of the ascending sap current due to transpiration is reduced to its minimum at this part of the 24 hours, the large volume of water in the trunk and bud has a specific heat about five times greater than that of the solid soil particles. This offers to the outside cooling influence a resistance which is of the utmost importance to the tender tissues at this critical period.

During this frost period of February 19 both tree thermometers registered temperatures safely above 50° , which temperature the writer has found to be the minimum point for growth of the date palm leaves (17).

A sharp decline in the growth rate of the leaves of this tree for both February 19 and February 20 indicates that the zero point of this rather sensitive variety had been closely approached; yet the growth of 1.5 2 mm. on February 20 shows that the actual zero point, as applied to growing tissue, had not been reached with the

interior temperature at 52.7° . This is an example of the importance in growth-temperature studies of knowing the actual temperature maintained within the growth-center, although such knowledge is usually difficult to obtain.

Another conclusive proof that the lower curves of the interior temperatures are in large measure due to the cooling influence of the ascending sap current lies in the records of April 21 to 27 (Table IV and fig. 6) where on the 23d, 26th, and 27th particularly, the midday temperature shown by the lower trunk thermometer was 3° to 5° lower than that of the upper one and on the latter date actually coincided with the soil temperature. While the retarded effect of the cool night air has an undoubted influence in bringing about the lowered temperature of the interior at midday, the records of February 19 show that the interior of the palm at the growth center is much more sensitive, and more quickly reacts to external cold than does the trunk interior near the ground. Affected only by the cooling of the night air, then, the upper thermometer, after allowing time for the lag, should show a lower midday temperature than the thermometer 2 feet from the ground. This has occurred only a few times; once on February 19 under the influence of the sudden drop to 25° , which was more than sufficient to overcome the influence of the soil water at 58° .

The influence that could keep the lower trunk center at a lower temperature than the upper one at midday would be that of the ascending sap current, which would be most active during the period of greatest transpiration. Although the growth-center temperature is no doubt lowered by the same influence, the ascending sap reaches it later, after having absorbed more heat from the surrounding tissues. Though no observations have been made on the hourly transpiration rate of the date palm, the studies of Briggs and Shantz at Akron, Colo. (3), show that for a variety of plants, but especially wheat and rye, the maximum of the day's transpiration occurs later than midday, often between 2 and 3 p. m. It will be noted from the Tables I to VI of interior temperatures of the date palm that the minimum recorded by both the upper and the lower thermometers is usually at a point about midway between 12 noon and 6 p. m.

April and May, being unusually cool, did not afford the usual examples of high temperatures, but April 29 and 30 and May 1, 2, and 3 gave maximum air temperatures of from 95° to 99.5° F. with daily means from 78.5° to 82.5° (Table V). Yet the minimum interior temperature of the Zaheedy tree by the lower thermometer at 3 p. m. (the upper one was broken) was 71.6° on the 29th and 72.5° on the 30th, with the soil at 70° and 71° . During the first three days of May, with air maxima reaching 98° and 99.5° , this interior temperature was only 2.5° to 2.7° above that of the soil. On May 2, with the maximum air temperature 99.5° , the interior of the Zaheedy touched a minimum of 73.4° , a protective cooling of 26.1° . At the same time the growth center of the Maktoom tree gave a minimum of 76.1° , giving these active cells a cooling effect of 23.4° below the air maximum. For this entire period the temperature curve for the lower Zaheedy thermometer was below the daily mean air temperature. With two exceptions, the growth center temperature of the Maktoom was also below it. This gives another proof of the cooling influence of the ascending sap current.

Here, again, the principle of the high specific heat of water plays a most important part, conversely to that when air temperatures are below soil temperature; water is slow to cool or give up its heat, but is also slow to absorb it, hence serving as the great factor in stabilizing temperatures of the tissues of the growth center.

A LATER SERIES OF RECORDS

The most convincing proof that the temperature of the soil water is the chief governing factor in regulating the interior temperature of the trunk during hot weather is found in records secured in the spring of 1923 by the study of a large male palm known as the "Mosque Male" in the collection at the Indio date garden. This tree was a 9-year-old seedling at the time and 11 feet high to the top of the bud, with a trunk diameter of 2 feet at 4 feet high. Doubtless the greater size and more mature age of this tree rendered it less susceptible to changes in air temperature than the smaller trees of the earlier tests, also the greater extent and deeper penetration of its roots would give a different reaction in relation to soil temperatures. In February a thermometer was placed in the trunk at 4 feet high, the bulb reaching to the center of the tree, and another in the near-by soil 2 feet deep. It was not expedient to place a thermometer in the growth center of this valuable tree.

The records obtained showed that the trunk temperatures were higher than those of the soil by 2° to 4° F. in the early morning, but approached most closely at about 2 to 3 p. m., occasionally coinciding. On May 5, a very hot day, the first of the season, trunk temperatures from 10 a. m. to 4 p. m. were lower by 1° to 1.75° than the soil temperatures at 2 feet deep. On May 7 another soil thermometer was installed at a depth of 3 feet.

It at once became clear that the lower trunk temperatures were due to soil water drawn from a greater depth than the 2-foot layer. Table VI, for May 5 to May 11, comprises a period of unusually high temperatures for the early part of May with the air of desert dryness, according to Indio date garden records, the relative humidity at 2 or 3 p. m. being only 10 to 15 per cent.

The trunk temperatures declined from about 8 a. m. until 2.30 or 3 p. m., the period when leaf transpiration is presumably the greatest. Here the trunk temperature fell below that of the soil at 2 feet, and at times approached closely to that of the soil at 3 feet, where the heavier feeding roots of a tree of this age are found. Evidently the heavy demand made upon the roots for water at the mid-afternoon period carried the sap up through the trunk at such a rate that there was comparatively little lowering of the temperature at which it left the soil. At what actual temperature this sap reached the growth area of the bud, 5 or 6 feet higher, must be inferred from the records obtained in 1918 from smaller trees. Table IV shows that when the soil temperature at 2 feet was 71° F., the midday temperature in the growth area was 3° to 5° higher. A 6° increase in sap temperature for the Mosque Male (from soil at 2 feet to the growth area of the bud) would give this region a temperature around 80° at the time when the air maximum was 108° to 110° , or a protective temperature difference of from 28° to 30° . It must be remembered that the basal growing points of all incompleated leaves (of which there may be 6 or 8 in process of elongation at once) center in this

area of cell division. A fruiting tree has in addition the bases of 6 to even 15 fruit-bearing stalks which continue their elongation well into the summer; also, the nourishing of the rapidly growing bunches of fruit must be provided for. The work performed in this huge growth center is therefore very great and the temperature protection afforded by the ascending sap current during the intense heat of the desert afternoon is of the greatest importance.

While attributing a most important influence to factor (3), the ascending sap current, it is evident that that can only be effective within the protecting and insulating influence of the protecting envelope and the fibrous outer tissues of the trunk proper.

Without these the sap current would be brought to the air temperature before it could reach the growth center. It might be compared to the attempt to convey hot water or steam from a heating boiler to a radiator in a distant building without the insulating coverings and inclosed conduits which heat engineers provide for such service.

Unusual space is given to this topic because in the idea of the regulating value of the ascending sap current in determining the temperature of the phyllophore of the palm there appears to be a heretofore unrecognized principle in plant physiology, of first importance in the date palm, the *Washingtonia*, and other desert endogenous plants, and not to be ignored in the economy of exogenous plants, both woody and herbaceous.

DISCUSSION

EQUALIZATION OF TEMPERATURE IN THE GROWTH CENTER BY TISSUE INSULATION

The idea of the thermos bottle preserving the temperature of either cool or hot contents was anticipated by the date and other palms, though unrecognized all these years. In fact this idea clearly explains the wonderful adaptation of such palms to the extreme temperature ranges of their environments. The heavy layer of fiber surrounding the trunk of the date palm is only relatively less efficient than the arrangement of outer and inner flasks with a nonconducting vacuum zone between. The excessive heat of the midday air and the chill of the frosty night alike penetrate but slowly toward the tender embryonic cells in the bud. The strong volume of the sap current, absorbed by the roots at deep soil temperatures, ascending through the intricate vascular system of the trunk, is able to preserve most of its low temperature until it is dispersed in the leaves, because of the low conductivity of the protective envelope, the layers between it and the surrounding air. Thus the stabilization of temperature in the growth center of the date palm is established.

It is not too much to affirm that but for this protective adaptation to the extreme heat conditions of its environment, the death point for the growing tissue of the date palm would be reached nearly every year in the Sahara, and frequently in its adopted home in southern California. This would hold equally true of the *Washingtonia*, the one native palm of California, in which the writer has observed temperature differences between the growth center and the air similar to those of the date palm. The survival and majestic growth of the Canary Island palm, *P. canariensis*, at the Furnace Creek Ranch in the Death Valley of California, with almost the world's highest heat record, can be explained on no other hypothesis.

THE TEMPERATURE OF RESPIRATION

Without means for definite determination, it must nevertheless be assumed that to the warming effect of the daily air maxima there should be added some amount of heat developed by the activity of the enormous bud, which comprises not only the region where new organs are in formation but also the basal zones of all leaves still in process of elongation, sometimes six or eight in number. Jost (9, p. 398-401) mentions "growing points" along with germinating seeds and inflorescences as exhibiting temperatures higher than that of the surrounding air, but lays special emphasis on the high temperatures observed in the inflorescences of *Palmeae*, *Cycadaceae*, *Victoria regia*, and *Arum italicum*. He adds in the supplement (10): "The heat produced is thus not a protection against frost."

MacDougal (14, p. 57) by inserting thin-bulbed thermometers "in the tissues of joints of cacti from which new shoots were arising" observed temperatures at times 8° or 9° C. above surrounding air. The writer is unable to find any record of such temperature tests of the buds or growth centers of palm trees. Of the comparative temperatures illustrated in the five figures previously referred to, the greatest actual difference between the growth center and the surrounding air was 28.8° F. at 6.10 a. m. on February 19 (Table I). For protection to the growing tissue this was most important, since in the adjacent garden buds of many deciduous fruit trees were destroyed by that freeze. This was at a time when the night leaf growth was only 3 mm., hence the cell activity and respiration must have been very slight and the generation of heat from this source negligible.

The extent to which respiration becomes a factor in the interior temperature of the phyllophore may well be left to further experimentation.

It has been shown in another line of experiments that the leaf elongation of the date palm is made in the night and that growth entirely ceases in direct sunlight. But it has also been demonstrated that by excluding all light from a date plant at midday, a rate of growth is quickly resumed similar to that of the night hours. This would involve the activity of the meristem cells of the growth center following a maximum of respiration. Any rise in temperature, contrary to the usual downward direction of the temperature curve at this hour, could then be credited directly to the respiration in progress.

With the aid of a recording auxanometer and a recording resistance thermometer, it should be possible to coordinate almost to a minute the resumption of the suspended growth with any rise in temperature.

TEMPERATURE CONTROL OF THE GROWTH CENTER OF ENDOGENS

A survey of the literature on the temperature relations of plants fails to show recognition of the following four vital facts of plant physiology which are brought out in these studies of the date palm:

(1) The existence in an important plant group of what may be termed "giant buds" or growth centers, within which are in progress simultaneously (and in the date palm, at least, perennially) the basal or intercalary growth of leaves and flower spathes, and the apical growth of the trunk.

(2) Stabilized temperatures within this growth center, with a decided gradient between them and those of the surrounding air, higher or lower, as the case may be.

(3) An insulating zone of tissues of low conductivity, surrounding the growth center, and resisting the penetration of air temperatures from without and the escape of heat from within; a stabilizer.

(4) The stabilizing influence of the ascending sap current, tending to hold the growth center temperatures within a few degrees of the temperatures of the soil penetrated by the plant roots.

Pfeffer (19, pp. 379, 381) appears to have had an understanding of the influence of the ascending sap current in exogenous trees.

Rameaux (20) recognized the influence of "organic action" as "vital heat" in the plant, but attributed the plant temperature chiefly to "the influence of the climate, acting in two different manners—(1) directly upon the plant organs exposed to the atmosphere; (2) upon the soil and consequently upon the sap drawn up by the plants."

The direct action of the sun's rays was considered the strongest influence in governing plant temperatures and he recognized the effect of the slow penetration of the heat through the badly conducting wood.

Thus the alternations of the day's heat and the night's coolness did not reach the center of the trunk of 0.5 meter diameter until after a lag of 15 hours, or even 24 hours.

However, with the exogenous trees which were under observation, such temperature phenomena could have had little stabilizing influence on the meristematic tissues and none is claimed by this author; for he records that "during the warmest days of April, a branch of a poplar, 4 cm. thick, showed in the central strata a temperature at noon which was 8°, 10°, or even 13° C. higher than the surrounding temperature."

Of the influence of the sap current, Rameaux says: "The ascending sap increases or diminishes the temperature of the parts which it traverses according as these parts possess a temperature lower or higher than that of the sap itself."

Much importance is attached by this author to the transpiration rate of the foliage in enhancing the flow of sap through the trunk and branches and so increasing its cooling power as it meets the heat penetrating from the surface. In support of this idea he submits a table of the interior temperatures recorded in two trees; in the first period, growing normally, the temperatures of the two trees during various hours of the day compared very closely. During the second period, with the second tree dead and presumably not transpiring and without the ascending sap current, the dead tree showed during the afternoon temperatures of from 7° to 10° higher than those of the normal tree cooled by the ascending sap. In the third period, with tree No. 1 shorn of all its top, hence with no ascending sap current, its interior temperature compared closely with that of the dead tree.

DeCandolle (A. P.)⁷ (6) *Physiologie Végétale*, is cited frequently by both Pfeffer and Rameaux. DeCandolle recognized the impor-

⁷ Citations from DeCandolle, Aug. Pyr., (6) in *Physiologie Végétale*; (p. 879) Schoepff (2) *Naturforscher*, 23 stl., p. 1-37. Halle, 1788; (p. 880) Hermstaedt (2), *Mag. d. Gesellsch. naturf. freunde in Berlin*, 1808, p. 316; (p. 879) J. Hunter, (1) *Philos. trans. for 1775 et 1778*; Journ. de phys., 9, p. 294; 18, p. 12 et 216.

tance of the ascending sap current in regulating the temperature of the trunk interior and stated that "Buffon was the first author to observe that when we cut trees in the winter, the interior part of the trunk appears to be warm, especially close to the center, * * *."

He cites Hunter (p. 879) as the first to try to explain the phenomena by placing a thermometer in a hole bored in the trunk of a large walnut tree, where he obtained in autumn, temperatures 2 or 3 degree above the surroundings. He was followed by Schoepff of New York (p. 879) and Bierkander of Sweden, confirming the earlier work.

DeCandolle cited John Schöpf (21), who published "Ueber die Temperatur der Pflanzen," in *Naturforscher*, Halle, 1788. This is a record of observations made at New York in 1783 on the temperatures within the trunks of several species of trees, black, red and white oak, wild cherry, chestnut, and beech, compared with those of the surrounding air. He observed distinct gradients between tree and air temperatures, notably one morning with the air at 22° at 7.45, when the interior of a 3-foot chestnut showed 36°. Schöpf is quoted as having "repeated and enlarged" Hunter's experiments. These records, probably the beginning of the study of plant physiology in the United States, he interpreted as proving that plants generated in themselves a vital heat analogous to that of warm-blooded animals. He failed to associate the higher interior tree temperatures with the ascending sap current and soil temperatures.

DeCandolle further records that Pictet and Maurice at Geneva, by the use of several thermometers, some in the trunk of a large chestnut tree, others in the soil at various depths, arrived at a true solution of the problem:

They noticed that the variations in the internal temperature (in the trunk) corresponded plainly with the temperature given by the thermometer placed in the soil at the depth of 4 feet, thus in the vicinity of the roots of the tree.

Hermstaedt (p. 880) is quoted as observing a temperature of plus 1° R. (34.25° F.) within a tree when the air temperature was minus 10° R. (9.25° F.). DeCandolle sums up this part of the problem as follows:

In bringing these facts together, and especially the last ones mentioned, Rumford's theory about the ascension of the sap helps us to appreciate the problem dealing with the internal temperature of plants as I have attempted to explain in 1805 in the "Principes de Botanique" in the introduction to the *Flore Française*. * * *

The water absorbed by the roots ascends vertically in the trunk. This water shows the temperature of the soil at the middle depth of the tree roots; it is consequently warmer than the atmosphere in the winter and cooler in the summer.

The insulating effect of the bark and the outer layers of the trunk in conserving the heat of the ascending sap is recognized in the following paragraph:

Therefore, while the ascension of the sap tends continually to bring the temperature of the center of the trunk to an equilibrium with that of the soil, the whole structure of the woody body of the trunk and especially that of the bark, prevents the temperature from reaching an equilibrium with the surrounding air.

A summary of these observations on the interior temperatures of trees, dating back from DeCandolle in 1832 to Schöpf in 1783, and Hunter in 1775 shows the following points generally accepted:

(1) The paramount influence of the air temperature and, in sunlight, the sun's rays incident upon the surface of the trunk and branches, in controlling the interior temperature of the tree.

(2) Balanced against the first, the influence of the ascending sap current, deriving its temperature from the soil at the depth of the greater mass of the absorbing roots, hence subject to but moderate fluctuations. It was recognized that this sap current might exert a cooling or a warming influence on the general body temperature of the plant according to whether it was of a lower or higher temperature than that derived from the air; becoming a cooling influence in the summer, a warming one in the winter.

(3) Interposed between the external air and the trunk interior, DeCandolle recognized the insulating value of the bark and the woody trunk, resiting the penetration of the exterior heat or cold on the one hand; blanketing and conserving the temperature of the ascending sap on the other.

(4) The importance of leaf transpiration in inducing the flow of the sap current was recognized when the top was removed from the tree in Rameaux's experiment, the rise of the trunk temperature when exposed to the sun being attributed to the absence of the cooling sap current ascending at soil temperature.

Influence of the interior temperature on the growth centers and cambium is nowhere suggested, nor was there any recognition of the giant buds which characterized such genera as palms, yuccas and Dracenas, nor of the specially insulating layers which surround such buds in the date palm.

LIMITATION OF THE GEOGRAPHICAL RANGE OF THE DATE PALM BY TEMPERATURE

The geographical occurrence of the date palm must be considered under two categories.

(1) THE ARTIFICIAL OR PLANTING RANGE

The artificial or planting range in which the date palm will survive is a much wider one than the range of fertile seed production and must be limited by such a degree and duration of cold as would enable frost to reach the meristematic tissues of the growth center. It has been shown in previous pages that growth was not wholly checked by minimum temperatures above 21° or 22° F. of short duration, but many hundreds of palms in the Coachella Valley of California survived minimum temperatures of 13° to 15° January 7 and 8, 1913, with only a severe killing of the leaves. At the United States Experiment Station at Sacaton, Ariz., this same cold spell gave minimum temperatures, January 6, 9°; January 7, 10°; and January 8, 11°; with similar results, a severe freezing of leaves, but the growth center unharmed. In San Antonio, Tex., previously referred to, mature palms have survived minimum temperatures of 4°, though all foliage was killed. The short duration of the severe cold would have had little effect on the soil temperature penetrated by the roots and the latent heat of the sluggish sap current must have been able to overcome the penetration of cold toward the bud.

(2) THE NATURAL OR SEED PRODUCTION RANGE

The natural or seed production range of the date palm would be within a climatic area which would permit the production of viable seeds for the propagation of the species. This lies far inside the area

where the palm may survive if planted. There are limits to the laying down of the flower spathes, which would be governed by the temperature maintained in the phyllophore during the season of greatest heat. This would probably involve also a certain optimum intensity of the sun's rays, governed both by the angle of incidence and by the amount of watery vapor intervening, for the relation between photosynthesis and growth in the date palm is to a great degree a daily and direct one instead of the seasonal one that it is largely in exogenous trees. But throughout, the temperature of the sap current, under the insulation of protective layers of fiber, will be found as regulatory as a thermostat.

DUAL TEMPERATURE REQUIREMENTS OF THE DATE PALM

Thus the date palm has two very distinct temperature requirements. The first is effective insulation and protection of the tissues, while in the embryonic stage, against extremes of heat and cold. The second is high temperatures from both the surrounding air and the radiant heat of intense sunshine for the promotion of photosynthesis and the building up of a fruit crop with a high sugar content.

SUMMARY

The date palm is the most resistant to extremes of heat and cold of any member of the palm family. As a species it has survived without permanent injury extremes of temperature from 4° to 125° F. This wide range of adaptability is believed to be largely due to the morphological structure and physiological action outlined in the following paragraphs:

The date palm, along with other members of the palm family, has its active growing tissue centralized in a giant terminal bud or phyllophore from which are in progress simultaneously the basipital or intercalary growth of leaves and flower spathes and the elongation of the trunk and its thickening to the established diameter.

Within favorable temperature limits, and with sufficient water supply, the growth of the date palm is continuous throughout the year, the curve of mean daily leaf elongation being closely parallel to the curve of mean daily temperature. Growth may be continued when the minimum air temperature of the day is several degrees below the freezing point, provided the maximum temperature of the day is well above the growth zero point, 50° F.

Thermometers inserted in date palm growth centers show a stabilizing of temperatures with a marked gradient between them and those of the surrounding air, but with a striking correspondence with the temperatures of the soil strata penetrated by the palm roots.

The daily range of these interior temperatures is slight and rarely exceed 7 or 8°, but with an inverted curve, relative to the air temperature curve, highest at about sunrise, lowest at from 2 to 4 p. m.

The difference of the interior temperature from the surrounding air has ranged from 26° warmer on the coldest morning observed, to 32° cooler on the hottest day.

This protective stabilizing of the temperatures of the meristematic tissues of the date palm is believed to be due to two principal factors:

(1) *A protective envelope surrounding the growth center*, of a highly nonconducting and insulating nature, composed of the thick, fibrous,

overlapping leaf bases, with the accompanying fibrous sheath and lower down the outer zone of the trunk proper. Through these layers the air temperatures, cold or hot, penetrate very slowly and with considerable loss.

(2) *The ascending sap current*, with a temperature acquired from the soil from which it is drawn by the roots. Under the insulation afforded by the protective layer and the outer portions of the trunk, the sap is able to reach the growth center at a temperature varying but a few degrees from that at which it left the soil, and so is able to neutralize much of either cold or heat, as the case may be, that has penetrated from without.

It is believed that in the discovery of this stabilizing of the temperature of the growth center of the date palm is disclosed a principle heretofore unrecognized in plant physiology, and one of vital importance to palms and other endogenous plants in desert environments in enabling them to survive the extremes of their temperature exposures.

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THE INHIBITIVE EFFECT OF DIRECT SUNLIGHT ON THE GROWTH OF THE DATE PALM¹

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INTRODUCTION

A study of the habits of the growth of the date palm reveals an adaptation to the conditions of desert climates to which attention has only recently been directed (5, p. 3, 4).²

The lower minimum temperature point for growth has been treated (6), leaving still much work to be done on the optimum and maximum cardinal points for growth. The protective temperature regulation of the growth center and its influence on other temperature reactions, making growth possible the whole year, has been shown (7). The reaction of the date palm and a number of other palm genera to light conditions are considered in the present paper.

The date palm, along with many other palms, has a diurnal period of leaf elongation, in darkness or in the absence of direct sunlight, alternating with a complete cessation of growth in bright sunlight. This diurnal alternation of growth and rest, occurring inversely to the exposure to direct sunlight, is discussed in the following pages.

The literature on the growth habits of the date palm is very meager and the most of it is lacking in definiteness. Branner (1, p. 481) states:

In conclusion I find: (1) That all fronds and spadices originate at the center of the phylophore; (2) that the fibrovascular bundle division continues to grow until its frond reaches maturity; (3) that the growth of a palm trunk continues as long as the bundle divisions of the part are in active connection with living fronds, and no longer; and (4) that the growth of palms is therefore an internal growth, and the term "endogen" is not a misnomer as far as palms are concerned.

The inference that the entire growth of the leaf is made within the "phylophore" (correctly phyllophore) may be drawn from this, but is not positively stated.

A study of date-palm growth involves the questions: (1) In what regions of the plant is the growth made? (2) What relation does growth bear to temperature? and (3) What relation does growth bear to light and darkness?

The experimental approach to these questions calls for methods adapted to the particular structure of the date palm which is distinct from exogenous plants generally and also from many other genera of palms.

TERMINAL LEAF GROUPS

The leaves of the date palm when full grown are distributed around the axis very accurately according to the system of phyllotaxy of the genus; but in emergence from the bud they appear to follow growth impulses or waves in that from three to five leaves emerge together in close contact and with the perfectly formed

¹ Received for publication Sept. 26, 1924; issued October, 1925.

² Reference is made by number (italic) to "Literature cited," p. 468.

pinnae closely appressed, maintaining a growth rate as one individual. The form suggests an acutely sharpened stake for which the writer proposes the name *palus*, from the Latin *pālus*, a stake.

This palus or synchronous group of leaves may be extruded to a length of 2 or 3 feet before the new growth from within separates them and they assume an individual rate of elongation.

EXPERIMENTAL DATA

METHODS OF THE EXPERIMENT

In order to determine the various questions of growth in relation to light and temperature, seven seedling trees at the United States Date Garden at Indio, Calif., were selected for observation.

As the palus of new leaves emerged from the buds of two of these selected individual leaves were marked into 1 cm. and 10 cm. divisions, in order to learn whether any further elongation was made. The length of unexpanded pinnae was also recorded to learn whether they had attained their full length. In no case was any further elongation of light-exposed portions of either rachis or pinnae observed. A varying portion of the leaf was pushed up daily from the heart of the bud, the movement of which will be discussed later. The depth at which actual elongation of the tissue was taking place could only be surmised from external observations.

Next a discarded male tree about 5 years old was dissected in order to trace the elongation to its source. A sector of one-third of the circumference was cut out to 10 inches below the top of the bud, and two-thirds of the radius toward the center. Without invading the very center of the bud, portions of the bases of several of the younger leaves were reached where the tissue was ivory white, the fibrovascular bundles yet unhardened, and the whole substance easily broken with a square brittle fracture. While these dissections were being made, the admirable binding quality of the fibrous older sheaths was at once apparent. The whole central part of the bud was in a state of tension from the turgescence of the growing parts within. A distinct popping sound could be heard as the tensely strained layer of "leaf" or sheath fiber was severed with a quick cut of the knife and the pressure within relieved. If the leaves in the bases of which lignification had not taken place were not supported at once, they were broken by the slightest swaying by the wind or overbalanced by their own weight. Ten-centimeter portions were marked in centimeters on the succulent exposed portions of five such leaves, and these again marked in millimeters with a needle point.

Here, finally, proof of elongation was secured. The millimeter spaces in the lower portion of the marked areas were found to be farther apart in the daily observations, though the elongation was slight and continued only three days. The slight pushing up of the leaf from below showed that only the upper portion of the elongating area had been exposed. It seems probable that exposure to the air checked the elongation after three days, and that the severe cutting brought the whole growth to nearly the zero point. Subsequent experiments, where holes were bored to the center of the trunk, determined that this pushing up of tissues was quite active 18 inches below the bud crown, and at 2 feet below there was still a discernible action. This mode of growth is referred to as "basipetal" by Jost in the following paragraph: (4)

Frequently we meet with another type of leaf expansion where the apex at once passes into a state of rest. This is the case in many lianes where specially formed apices, fulfilling particular functions, are produced long before the rest of the lamina is completed. The elongation is basipetal also in the long leaves of monocotyledons, owing to the development of an intercalary growing zone at their bases.

Attention has been called (?) to the fact that all of this basipetal growth was so deeply seated in the bud as to be in complete darkness; quite the reverse of the mode of terminal growth of endogenous shoots like the bamboo or in the twigs of exogenous shoots like the apple or the pine, where cell division must go on within translucent tissue, or in very small twigs in nearly full sunlight.

PERIODICITY OF LEAF ELONGATION

Several trees of different ages were selected for study of their leaf growth, five of the newest central leaves being designated by letters, and the trees by their place numbers in the blocks.

The method of recording the advance in growth was by driving stakes, or for taller trees by erecting little towers. Upon these were placed carefully leveled crosspieces, over which a steel square enabled the record marks to be made on the ribs of the leaves with a thin-bladed knife. A day's growth of as little as 1 mm. could be accurately recorded. At first observations were made morning and evening, but the fact was soon disclosed that with occasional exceptions the expansion or pushing up from below took place almost wholly at night.

As it was not feasible at first to keep these records in close coordination with sunrise and sunset, the observations being made about 8 a. m. and 4 p. m., only the general facts of the relation of growth to light and darkness were brought out. Two facts, however, soon gained prominence, in addition to the main one (that the chief growth is made in darkness). The first was that intermittently some growth was made during daylight hours. Whether this was a holding over or prolongation of the night growth, or was due to conditions occurring during the day, could be shown only by securing continuous or auxanometer records. The second fact noted was that certain leaves, and on some days several of them, were actually shorter at night than when the morning reading was made. This interesting phenomenon, which has been observed in the growth history of a variety of plants and is attributed to decreased turgescence of the cells, will be made the subject of further study.

AUXANOMETER DATA

In April, 1918, an auxanometer was installed by making use of an anemometer clock having a horizontal recording cylinder revolving once in six hours. The spirally threaded spindle for carrying the record cylinder laterally to receive the wind velocity record was discarded, leaving the cylinder its rotary motion in a fixed position on its axis, and a pen-carriage was constructed to move from left to right at the pull of the wire actuated by the leaf growth. This clock in its case, was placed on a rigid stand by the side of the tree to be observed.

Since the midrib of the date leaf was quite rigid and pushed upward by a positive expansion below in the heart of the bud, an

upward pull on the fine piano wire fastened to the rib a few inches from its emergence from the bud gave a direct index of the growth being made. A piece of coiled wire tubing fastened rigidly to the palm trunk below the record leaf and to the side of the clock case served as a conduit for the piano wire between its attachments to the leaf and to the pen-carriage. The pen-carriage traveled on two parallel horizontal guide rods, and the beginning of a record was placed at the left hand or distal end of the cylinder. The pull of the wire to the right in response to the growth was counterpoised by a small rubber band on the left, giving a positive and steady motion to the pen. At the same time the position of the band was adjusted to regulate the pen pressure on the record cylinder.

Although this mechanism develops some friction on the wire, and is lacking in the extreme delicacy usually essential to an auxanometer, the strong up-push of the growing date leaf and the rigidity of the rib probably compensated for these features; and the general stability of this device was a positive advantage in out-of-door conditions, with almost constant high winds to be withstood. It is obvious that with this arrangement the pen traces a smooth line around the cylinder and parallel with the base when no growth is made, while growth is recorded by a slowly advancing spiral. As the circumference of the

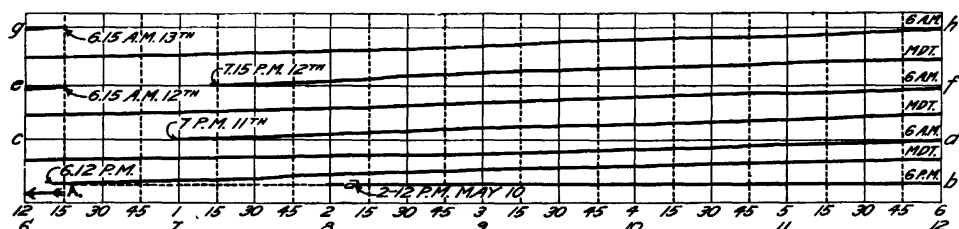


FIG. 1.—Auxanometer record of growth of date-palm leaf at the Government Date Garden, Indio, Calif., May, 1918

cylinder, making one revolution in six hours, is 12 inches, the cylinder must rotate past the pen one-sixth of 12 inches or 2 inches in one hour. Hence each five-minute period would be represented by one-twelfth of 2 inches or one-sixth of an inch on the circumference of the cylinder; while the longitudinal progress of the pen on the cylinder, actuated by the pull on the piano wire, would record the actual growth made.

This secures a fairly accurate recording of the beginning and ending of the growth periods, as well as a comparison of the growth rate during the different periods of the night.

When no day growth is made the pen does not advance and the pen-trace runs around the cylinder as a single line parallel to the cylinder heads at the point where growth ceased.

Between sunrise, when growth ceases, and sunset, when growth is resumed, the cylinder would make nearly two complete revolutions with the pen tracing the same mark.

Table I shows the daily growth, in millimeters, before and after midnight, with the time of beginning and ending from April 24 to May 20, inclusive. Figure 1 reproduces a tracing from one of the record sheets, most typical of three days' growth. (One should imagine this wrapped around the cylinder, with *c* and *d* contiguous and revolving in the direction of the arrow *A*.)

The horizontal lines *c-d*, *e-f*, and *g-h* show two revolutions of the drum during sunlight without registering growth. The heavy lines

ascending from left to right show night growth beginning at 6.12 p. m., 7 p. m., and 7.15 p. m. on April 10, 11, and 12, respectively, and ending 6 a. m., 6.10 a. m., and 6.15 a. m.

TABLE I.—*Daily growth of date palm before and after midnight, with time of beginning and ending from April 25 to May 20*

Days	Hour growth was resumed	Min-utes before sunset	Hour growth ceased	Min-utes after sunrise	Growth before mid-night	Growth after mid-night	Total growth	Excess of growth before mid-night	Excess of growth after mid-night
	<i>p. m.</i>		<i>a. m.</i>		<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
Apr. 25 to 26	5. 50	-----	6. 12	-----	8. 0	9. 0	17. 0	-----	1. 0
Apr. 26 to 27	5. 57	-----	6. 30	-----	10. 0	8. 5	18. 5	1. 5	-----
Apr. 27 to 28	5. 40	-----	7. 30	80	9. 5	10. 5	20. 0	-----	1. 0
Apr. 28 to 29	6. 50	15	7. 05	52	8. 0	11. 0	19. 0	-----	3. 0
Apr. 29 to 30	7. 12	-----	6. 42	29	7. 5	9. 5	17. 0	-----	2. 0
Apr. 30 to May 1	5. 20	108	6. 33	15	8. 0	10. 5	18. 5	-----	1. 5
May 1 to 2	7. 05	-----	8. 10	120	8. 0	10. 0	18. 0	-----	2. 0
May 2 to 3	6. 15	-----	9. 50	189	9. 0	13. 0	22. 0	-----	4. 0
May 3 to 4	5. 55	85	8. 35	223	10. 5	12. 5	23. 0	-----	2. 0
May 4 to 5	6. 12	69	6. 55	50	12. 0	12. 0	24. 0	0. 0	0. 0
May 5 to 6	7. 05	-----	9. 00	170	8. 5	11. 0	19. 5	-----	2. 5
May 6 to 7	6. 25	-----	7. 30	80	10. 0	10. 5	20. 5	-----	0. 5
May 7 to 8	4. 32	161	8. 35	145	12. 5	10. 0	22. 5	2. 5	-----
May 8 to 9	4. 52	148	6. 45	33	11. 0	8. 5	19. 5	2. 5	-----
May 9 to 10	5. 00	148	10. 00	220	11. 5	9. 5	21. 0	2. 0	-----
May 10 to 11	6. 10	70	5. 47	21	8. 0	6. 5	14. 5	1. 5	-----
May 11 to 12	6. 55	32	6. 15	-----	9. 0	9. 5	18. 5	-----	0. 5
May 12 to 13	7. 16	10	6. 15	-----	9. 5	10. 5	20. 0	-----	1. 0
May 13 to 14	6. 50	30	7. 50	110	8. 5	12. 5	21. 0	-----	4. 0
May 14 to 15	7. 00	20	6. 12	8	10. 0	13. 0	23. 0	-----	3. 0
May 15 to 16	5. 22	125	9. 00	180	12. 0	10. 0	22. 0	2. 0	-----
May 16 to 17	7. 10	20	6. 45	36	10. 0	10. 0	20. 0	0. 0	0. 0
May 17 to 18	6. 43	51	8. 03	60	10. 0	10. 0	20. 0	0. 0	0. 0
May 18 to 19	6. 23	75	8. 05	102	9. 0	11. 0	20. 0	-----	1. 0
May 19 to 20	6. 15	75	7. 52	110	12. 0	9. 5	21. 5	2. 5	-----
May 20 to 21	6. 12	78	8. 28	148	10. 0	12. 0	22. 0	-----	2. 0

Figure 2 shows the daily growth for nine successive days (May 10 to 19) plotted to a uniform scale; a single line for each night's growth ascending from left to right between the horizontal lines of no growth during sunlight. The vertical lines show the hours of growing time before and after midnight, with the lines of heavy dashes indicating approximately the time of sunset and sunrise.

In a general way both Figures 1 and 2 and Table I show that the growth activity began at a variable time before sunset, progressed rather uniformly through the night and to a period of from a few minutes to two or three hours after sunrise. The excess of growth in the periods before and after midnight appears to depend on the prolongation of the growth period by the light being obscured by clouds and dust, so that normal growth may be regarded as nearly uniform throughout the hours of darkness. It should be noted also that the growth rate in obscured sunlight (for example, the evening of May 15 and the morning of the 16th) is invariably slower than that during full darkness.

Correlated observations on the weather conditions showed that when the air was clearest the beginning of growth was retarded the nearest to sunset, and growth ceased soonest after sunrise. With clouds over the mountain top to the west, obscuring the sunset, or with the air heavy with fine sand driven by winds from the same direction growth began at an earlier hour. After all-night winds which filled the air with dust, or with vapor present in the morning

air, growth was proportionally prolonged after sunrise. In other words, growth in the form of leaf elongation is for the date palm somewhat inverse to the light intensity. It is most active in darkness, nearly or completely checked in bright sunlight, and partial in obstructed light.

GROWTH REACTION TO ARTIFICIAL LIGHT AND DARKNESS

In order to be able to determine more definitely the relation of date leaf growth to light and darkness it was deemed necessary to provide for the exclusion of light at will. In order that otherwise normal growth conditions might not be disturbed, a folding dark cell was constructed which could be placed around a medium-sized palm in the field in a few minutes time. Four panels of light pine framework, each 5 feet wide by 12 feet high, were covered with black enameled cloth (the black surface within) to prevent excessive heating in the sunshine. Three of these panels were hinged together and when erected around the tree the fourth side was slipped into rabbeted

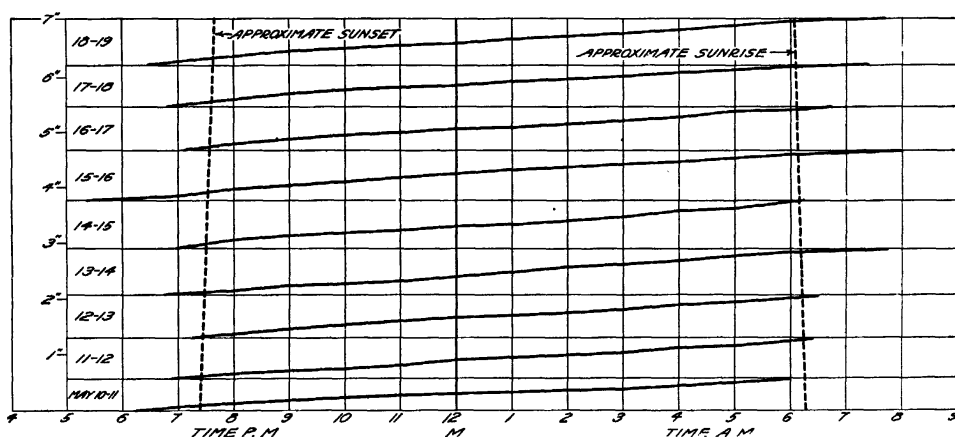


FIG. 2.—Daily and hourly growth of the leaf of Mactoum seedling date palm, plotted from auxanometer sheets, May 10 to 19, 1918

jambs provided to secure light-tight joints and held in place by hinged hasps. With the aid of high stepladders, the top, built like a box lid, was placed in position and also secured with hasps. Thus in a few minutes the date palm, with its spreading leaves drawn by cords into a more erect position (the only disturbance to its natural condition of growth) was inclosed in a completely dark cell or chamber 5 by 5 by 12 feet in size (300 cubic feet capacity). At first the auxanometer and stand were retained inside the dark cell, but it was found more convenient for observation to carry the recording wire through the house wall to the stand with instrument outside.

The first test was made on May 22. After a normal gain during the night of the 21st and 22d, growth had come to a complete standstill at 8 a. m., as shown by the auxanometer tracing, Graph I, Figure 3.

At 10.37 a. m., after 2 hours 37 minutes record of no growth, the dark cell was closed and remained in position till 4.55 p. m. As the actual auxanometer records are somewhat difficult to interpret, they have been transferred to graphs similar to the day and night records in Figure 2, but the time in hours is represented by one-half inch spaces, while the actual leaf growth is represented in the inch and

one-tenth inch vertical spaces. Graph I of Figure 3 shows above the base line the beginning of growth at 7.20 p. m. of May 21, with a rather even gain until 8 a. m. May 22, followed by the horizontal line of no growth to 15 minutes after the dark cell was closed. Active growth was recorded from this time till 5 p. m., five minutes after the dark cell was removed and the tree exposed to full afternoon light, when growth ceased.

From this the horizontal line till 7.25 p. m. indicates that the normal daylight dormant condition as to elongation was resumed in a very few minutes after the plant was exposed to the normal sunlight and continued until the usual night elongation commenced, which was 13 minutes before sunset. In Graph II this night growth is transcribed from 7.25 p. m. May 22 till 8.20 a. m. May 23, from which point a horizontal line of no growth continues till 10.15 a. m., 35 minutes after the dark cell was closed at 9.40 a. m. Apparently when the sunlight was shut out the growth did not start quite so promptly as on the previous day. Here the lack in sensitiveness in the recording mechanism is to be regretted, as a precise reaction time can be only

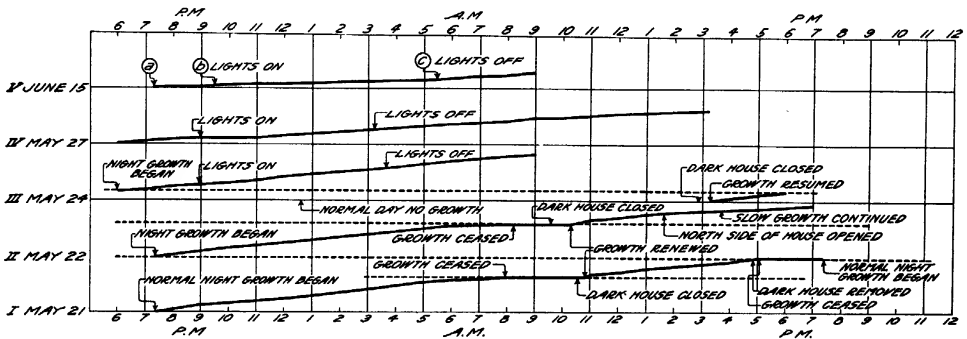


FIG. 3.—Effects of artificial darkness and incandescent light on date-palm growth at the Government Date Garden, Indio, Calif., May, 1918

approximated. On this day the dark cell was opened at 1.37 p. m. by removing the panel on the north side. With the top and three sides remaining in position the tree was still deprived of direct sunlight, receiving only the reflected north light, much of which was absorbed by the black interior walls, giving about the effect of a dull cloudy day. Instead of the growth being wholly checked, as when the dark cell was entirely removed and the tree exposed to full sunlight on the previous day, growth continued as shown by the graph at a less rapid rate than in complete darkness. Therefore exposure to full direct sunlight must be assumed as one of the conditions for growth cessation.

It has been suggested that in securing the resumption of growth within the dark cell at midday other factors than the complete darkness may have been involved, as a marked rise in temperature and an increase in the relative humidity. The reply would be that growth action was recorded before there was time for any very marked change in either temperature or humidity. Moreover the continuation of growth with the north side of the dark sell removed was made under conditions where but little gain in temperature could have resulted, and no increase in humidity, leaving the continuance of growth under partial lighting to be ascribed wholly to the absence of direct sunlight.

GROWTH UNDER ARTIFICIAL LIGHTING

In order to test the converse side of this problem it was next proposed to learn whether active growth in the night could be checked by exposing the tree to artificial lighting. Electric wires from a lighting circuit of 110 volts were carried to the dark cell and two 200-watt incandescent bulbs and two 60-watt bulbs installed, giving an illumination of 520 watts. At 2.53 p. m. May 24, as shown by Graph III, in Figure 3, the dark cell was closed around the tree and active growth was recorded in about 25 minutes. After this growth in darkness had progressed till 9 p. m., the lights were switched on and kept burning till 3.40 a. m., though the dark cell was retained in position till 9 a. m. The gain in growth was practically uniform through the entire period of nearly 18 hours. No checking of growth could be traced to the effect of this degree of illumination. A preliminary trial had, in fact, shown that the cell of 300 cubic feet of space was rather feebly lighted owing to the absorption of light by the black walls. Yet the same number of watts brilliantly illuminated the station office room, 22 by 16 by 10 feet high, or more than 10 times the cubical capacity of the dark cell. The dark cell was next lined with heavy white cotton sheeting and the lighting increased to six 200-watt bulbs, or 1,200 watts. The dark cell was closed at 5 p. m. May 27 and active growth was registered at 6 (Graph III). At 9 p. m. the lights were switched on. This was followed by no growth for two hours or until 11 p. m. At this point the growth was resumed and, until the lights were switched off at 3.15 a. m. May 28, continued at practically the same rate of growth as in full darkness until the dark cell was removed at 3.15 p. m. May 28.

This experiment was repeated in June under the supervision of Bruce Drummond. The lighting was increased to 1,800 watts and the dark cell was erected around the palm on June 15 and normal darkness growth began to be registered shortly after 7 p. m., as shown in Graph V, *a* in Figure 3. At 9 p. m. (*b*) lamps to the volume of 1,800 watts were switched on and continued until 5.30 a. m. of the 16th (*c*). The gain made during these eight hours of illumination was only 3 mm., but from the time the lights were switched off till 9 a. m. of the 16th (3.5 hours) a gain in the dark cell of 3 mm. was also made. In other words, the growth during the eight hours of intense illumination was at the rate of 0.375 mm. per hour, while that during the ensuing 3.5 hours of complete darkness was at the rate of 0.857 per hour. The illumination reduced the growth rate to 43.7 per cent of the rate in darkness. Consequently even so vivid an illumination as that given by 1,800-watt incandescent lamps within this small space of 300 cubic feet, while slowing down the growth rate to less than half, fails to give the complete check which is observed in the ordinary intense sunlight of this station. Evidently a difference in the quality of the rays from the incandescent lamps and those from the sun must be more important than their difference in intensity. The facilities of the Indio field station could take the work no further.

LABORATORY WORK AT WASHINGTON D. C.

A laboratory opportunity for testing the action of date palms under light of a different quality was afforded by the presence in one of the department photographic rooms of two powerful "Cooper-Hewitt"

mercury vapor electric lights working on a 220-volt direct current circuit. These have 50-inch lead glass tubes of "Type P," and are rated by the manufacturers as consuming a current of 385 watts each. Two seedling Thoory palms in 8-inch pots, having leaves in active growth, were selected and prepared for growth measurement by placing in the pot small standards carrying a horizontal gauge piece close to the midrib of the leaf to be recorded. Glass-headed steel ribbon pins were inserted for markers in the leaf rib in contact with the gauge.

A very slight degree of growth could thus be detected by the light being visible between the pin and the gauge, and this space was readily measured by the gentle insertion of one or more thicknesses of paper. Their total thickness was measured with a micrometer caliper gauged to 0.001 inch, but easily read to the half space. As in the artificial light tests at Indio, Calif., it was arranged to record first a period of active growth in complete darkness, then a period of exposure to the light, followed by a second period in darkness.

In preparation for the test the plants were placed in position between the mercury vapor tubes and well below them so that the reflectors would give them the full light. The room was completely darkened from 4.30 to 8 p. m., October 2. Then the 3½-hours' growth in darkness was measured, the pins put down to the gauge and the mercury lights switched on. After an exposure from 8 p. m. until 12 midnight the growth indicated was only the thickness of one sheet of paper (0.002 inch) on palm No. 1, and of two sheets (0.005 inch) on palm No. 2, which may have been a continuation of the darkness growth before the check. Left again in darkness till 8.30 a. m. of October 3, the gauge showed that the growth must have been quickly resumed, as 0.0245 inch of growth was recorded for each plant. The exposure was repeated the night of October 3 under the same conditions, but with a slight modification in time, the results confirming the first night's record. Table II shows the growth in thousandths of an inch for both nights.

TABLE II.—Growth of two date palms during the nights of October 2 and 3

Date and hours	Growth made by tree No. 1	Growth made by tree No. 2
	Inch.	Inch.
Oct. 2, 4.30 to 8 p. m.	0.0130	* 0.0080
Oct. 2, 8 to 12 m.0020	° 0.0050
Oct. 2 to 3, 12 m. to 8.30 a. m.0245	*. 0245
Oct. 3, 4 to 8 p. m.0250	*. 0250
Oct. 3, 8 to 11 p. m.0025	° 0025
Oct. 3 to 4, 11 p. m. to 8.30 a. m.0340	*. 0500

* In darkness.

° Under light.

The interpretation of these records, showing only a minute amount of elongation during the lighted period, seems clearly to be that after the early evening growth in darkness the exposure to the mercury vapor rays checked the growth wholly after a lapse of a few minutes. This reaction period is similar to that found to occur between darkness and sunlight action in the Indio tests.

The rays of the mercury vapor lights appear to have checked the leaf elongation as perfectly as did bright sunlight under Indian conditions, a result not attained with much more intense illumination by incandescent bulbs.

The next step is to compare the spectrum of the incandescent lights and the spectrum of the mercury vapor lights with the solar spectrum. Rays of the solar spectrum, which are lacking in the spectrum of the incandescent lights but present in the spectrum of the mercury vapor lights, must be the important rays in the physiological activity of the date palm, and presumably in that of the other palms which make their leaf elongation chiefly at night.

The subject of the effect of light rays of various colors on the growth of plants has occupied a great deal of attention for more than half a century, and many writers have contributed to its literature. Growing plants in light passed through glass plates of different colors or through colored solutions has shared the field with tests of growth under different portions of the prismatic spectrum. Electric-light culture has also received its full share of attention. The whole subject of the influence of light rays from different regions of the spectrum upon the physiological action of plants is too complex for more than brief consideration.

The writer is not aware, however, of any comparison of growth reaction having been made between light from ordinary incandescent bulbs and light from special illuminants, which either afford a close approximation to pure white light or exclude certain portions of the spectrum, as is done by the Cooper-Hewitt types of lamps.

The Smithsonian Tables 361 and 366 give the following wave lengths, in microns, for the standard colors (2):

Violet.....	0. 44
Blue.....	0. 46-0. 48
Green.....	0. 50-0. 52-0. 54
Yellow.....	0. 56-0. 58
Orange.....	0. 60-0. 62-0. 64
Red.....	0. 66-0. 68-0. 70

The same volume, Table 368, gives the visible spectrum as ranging from 0.644μ to 0.405μ and the ultra-violet from 0.384μ to 0.280μ .

R. D. Mailey, of the engineering department of the Cooper-Hewitt Electric Co., Hoboken, N. J., wrote as follows, in a letter dated October 9, 1918: "The lamps which you are using are made of lead glass, and you will have to keep this in mind, remembering that lead glass does not pass appreciable amounts of light below wave lengths of $3,800 \text{ } (\mu\mu)$."

According to the Smithsonian Table 368 (2) the ultra-violet rays begin with wave lengths of 0.384μ , which would leave practically no ultra-violet rays in the radiation from these tubes.

Mr. Mailey did not state the limit of wave length of these tubes in the direction of the red, but fortunately R. A. Steinberge, of the Bureau of Plant Industry, United States Department of Agriculture, had made a spectroscopic analysis of the rays from the identical tubes from which the results under discussion were obtained. These notes he kindly placed at my disposal. Five lines were observed with a hand spectroscope; a bright line in the violet at about 0.405 , and one in the blue, a faint line in the blue-green, a strong line in the green, and one in the yellow at about 0.578μ . Nothing was visible in the higher wave lengths.

W. W. Coblenz, in charge of light investigations in the United States Bureau of Standards, informally places the three light sources under consideration as follows:

Order of intensity

Ultra violet and violet:

- I. Sun.
- II. Cooper - Hewitt lead - glass mercury lamps.
- III. Incandescent lamps.

Red and infra-red:

- I. Incandescent lamp.
- II. Sun.
- III. Cooper-Hewitt lamps.

Summing up, on one side, the factors under which leaf growth occurs, and, on the other, those under which no growth occurs, the following result is obtained:

Date-palm photo-activity

Growth occurs:

Normal growth—

- a. In total darkness, at night or in a closed chamber.
- b. In chamber with incandescent lights, giving brilliant illumination.

Partial growth—

- c. During cloudy days.
- d. On clear days; with plant exposed to indirect light.
- e. In closed cell with 1,800-watt illumination.

No growth occurs:

- a. Under bright sunlight.
- b. Under Cooper - Hewitt lead-glass tubes.

Analysis of light conditions

Normal growth:

- a. In absence of all light rays.
- b. Under incandescent lamps. Complete spectrum but rays rich in red; no ultra-violet.

Partial growth:

- c. In absence of direct sunlight; proportion of spectrum unknown.

No growth:

- a. Under full solar radiation with intense illumination of atmosphere low in water vapor.
- b. Under Cooper-Hewitt lead-glass tubes; lacking red and orange; containing some yellow, full green, blue, and violet.

Jost (4, p. 127) concludes:

A comparison of observations derived from *all* the researches which have been made brings out the following points: (1) Only light of wave lengths between 770 μ and 390 μ is conducive to assimilating activity in green plants; these are approximately the same rays which are visible to us; (2) the assimilating effect of different rays is unequal, but still not in such a way that some are active whilst others lying beyond these are quite inactive.

Jost's estimate of available wave lengths, it will be noted, includes considerable infra-red.

For the position of the apex of assimilation, Jost refers to "Figure 27," adapted from Reinke (9, *pl. 1, fig. 6*); but in Reinke's original figure, while the apex is shown at about the Fraunhofer line B, efficient assimilation is shown down to 600 μ , which includes all of the orange, and considerable activity is shown down to the line D, which is about the upper limit of the rays from the Cooper-Hewitt tubes.

Palladin (8, p. 26), after discussing many intricate features of the problem, sums the matter up as follows: "Carbon-dioxide is thus seen to be decomposed most rapidly in green plants by the light rays between lines B and C."

In the date-palm experiment leaf growth is continued under brilliant illumination from incandescent lamps rich in red rays, while it is inhibited under Cooper-Hewitt tubes which emit no red rays, but are rich in rays of the shorter wave lengths.

That this is simply a rest period for this particular function, while other vital activities of the tree are in full progress is self-evident.

DAYLIGHT ACTIVITY OF THE DATE PALM

The next inquiry should be, What normal activities of the palm in sunlight are inhibited in darkness? According to general principles of plant physiology they would be: First, photosynthesis or the assimilation of carbon-dioxide; second, the greater proportion of the day's transpiration. From the records of leaf elongation in darkness it appears that in the case of palms such action is diametrically opposed to the daylight activities. The one begins where the other ceases. Whether the leaf elongation during the darkness or absence of direct sunlight is the result of cell multiplication with cell elongation, or of cell elongation only, is a question difficult of determination, but probably the whole cell constructive work of the phillophore is performed during this period.

The intense transpiring activity of date palms during heated days has been referred to in another paper by the writer (7) and this is accompanied by photosynthesis on a corresponding scale. There is evidently a close relation between the day's assimilation and the growth of the following night; which is proved by the close correlation between the daily growth curves and temperature curves. But this general relation would not explain the almost immediate growth reaction in response to artificial darkness produced in the middle of a bright day; nor the quick checking of growth when sunlight is restored. If the governing action of the stomates on transpiration is conceded, then by supposing that the stomates close in darkness or partial shade, the checking of transpiration must follow with increased turgescence of the cells in the meristematic region.

In this connection there may be much significance in the recently published studies of Gray and Peirce (3) on the reaction of the guard cells and the opening or closing of the stomates of barley, oats, rye, and wheat on exposure to bright sunlight or in its absence. These authors found that under the conditions at Stanford University, Calif., the stomates began to open on bright days soon after sunrise, reaching the fullest expansion from about 11.30 a. m. to 2.30 p. m., and gradually closed as the sun declined. When only portions of the day were bright, the curve of stomatal opening corresponded to these bright portions. On wholly dark days the stomates remained closed. They also found that if two pots of plants in similar condition, both showing the stomates partially open, were taken for experimentation, the plant in the pot subjected to darkness (with other conditions unchanged) soon closed its stomates. But the plant left in continued light (with other conditions unchanged) soon expanded its stomates completely. Reversal of the exposure of these two pots resulted in a reversal of the reactions of the plants.

Now, the conditions as to light and darkness were practically similar to those under which the Indio, Calif., date palms were observed. Where the date palms began growth the cereal plants of Gray and Peirce closed their stomates; where the date palms

fully ceased elongation at sunrise the grain plants showed steadily increased openings of the stomates; and where the date palms resumed growth under darkness supplied at midday, the grain plants closed their stomates.

In the absence of observations on this point it is reasonably safe to assume that the stomates of the palms react toward light in the same manner as those of the grasses.

While the original habitat of the date palm is only a matter of conjecture, in the cultivated state it is at home in the intense light and heat of the desert. Yet the intensity and quality of light under which it thrives, and especially under which it makes its best development of fruit, have heretofore received little attention. But evidence is not lacking that light conditions are second only in importance to temperature conditions for the growth of this tree. Walter T. Swingle (10, p. 58) anticipated this in 1904 when he wrote the paragraph on "Sunshine necessary for the date palm."

In the writer's study of the date palm in the northern Provinces of Sudan, a region of intense sunlight and dryness of the air, it was found that the native growers appreciated the necessity of the full exposure of the date-palm crown to the sun's rays. They have a system of allowing several offshoots or "daughters" to grow up to full maturity around the "mother" tree, thus giving a much larger number of stems to the acre than could be tolerated in America. Yet if one of these becomes overshadowed by a stronger growth the fact is at once recognized that its vigor and fruit production are seriously impaired.

SUMMARY

Date palm leaves are formed from the top of the phyllophore or growth center deep in the interior of the terminal bud, protected from light and from wide ranges of temperature.

The leaves, in groups of from three to five, called a palus are pushed forward by basipetal or intercalary increment, no alteration in their length occurring after they reach the light and assume their green color.

Normal growth, as manifested by the pushing up of the leaves from the growth center, is made chiefly in the time between sunset and sunrise, but also at a reduced rate in daylight, when direct sunlight is cut off by clouds. In full sunlight date palm leaf elongation entirely ceases.

Date-palm leaf growth may be induced in darkness obtained by inclosing the plant in a dark chamber, at any hour of the day. Partial growth may be induced in a similar manner by screening the plant from direct sunlight, but giving it exposure to north or reflected light.

Growth begun in darkness was continued at practically the normal rate when the plant was exposed to a battery of Mazda electric lights giving brilliant illumination.

When date plants after beginning growth in a dark room were exposed to the rays of Cooper-Hewitt lead-glass mercury vapor tubes, the inhibiting of growth was as prompt as in direct sunlight.

A spectroscopic analysis of the rays from Cooper-Hewitt lead-glass tubes shows that this light is confined to rays from the shorter wave lengths of the visible spectrum through violet, blue, green, and yellow to about the line D (0.578μ). The orange and red are completely absent.

It must be concluded, then, that the inhibiting of the date-palm leaf growth in intense sunlight of the desert regions is due chiefly to the action of rays of wave length from about $0.57\ \mu$ in the yellow to about 0.405μ in the violet end of the visible spectrum, but invisible ultra-violet rays probably assist in stopping growth.

Photosynthesis is most active in longer wave lengths from the line D at the end of the yellow to lines B and C in the red; thus growth is inhibited by light that has but little potency in photosynthesis and conversely carbon assimilation is favored by light that has but little ability in inhibiting growth.

Growth in absence of direct sunlight is apparently synchronous with the closing of the stomates, checking of transpiration, and increased turgescence in the meristematic tissue.

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AN IMPROVED METHOD OF COMPUTATION OF NET-ENERGY VALUES OF FEEDING STUFFS¹

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INTRODUCTION

The computation of net-energy values of feeding stuffs from data obtained by means of the respiration calorimeter has been explained in many publications by Armsby and Fries (1 to 9).² The general principle upon which these computations are based is well known and well understood. Essentially it involves the deductions, from the gross energy of the feed, of the entire expense of utilization of the feed energy for maintenance and production.

While the net-energy conception as promulgated by Armsby remains unaltered, the method of computation of net-energy values followed by Armsby and Fries in their later publications differs in detail from that used by them in the earlier reports, these differences representing improvements resulting from knowledge gained in the course of the experiments.

A further study of this computation in the light of present understanding has led to other improvements as here set forth.

DISCUSSION

The examples given will serve to illustrate the advantage of the latest modification, as compared with the earlier procedures.

Table I, Part A, quoted from Armsby's *Nutrition of Farm Animals* (8, p. 272), is given in that work as an illustration of the method of computing net-energy values for maintenance.

Two periods in which different amounts of timothy hay were fed are compared. The gain of energy is determined for each period by subtracting the heat production from the metabolizable energy. The difference in gain between the two periods, divided by the difference in feed eaten, represents the net energy per unit of feed. More specifically, the net-energy value of the hay is $2,028 \div 4.04 = 502$ calories per pound of dry matter.

It will be noticed that the same result may be obtained in this case by subtracting the heat increment per pound of dry matter of hay (433 Calories) from either the metabolizable energy per pound of dry matter of the hay eaten (935 Calories) or from the metabolizable energy per pound of dry matter of the added hay, these two values being identical. This occurrence is explained by the fact that the metabolizable energy per pound of dry matter of the hay is exactly the same in both periods which are compared. This is, however, rarely the case in practical experiments. The numerous sources of

¹ Received for publication Oct. 22, 1924; issued October, 1925.

² Reference is made by number (*italic*) to "Literature cited," p. 484.

error in the determination of the metabolizable energy³ cause more or less variation in the values per unit of feed in the different periods. Such variations may seriously affect the net-energy values computed by this method. If, for example, one assumes a difference in the metabolizable energy per pound of dry matter of the hay of only 50 Calories between the two periods, without changing the average metabolizable-energy value, the data of Table I, Part A, would be changed into those of Part B, or of Part C.

EXAMPLE 1

TABLE I.—*Computation of net-energy value of timothy hay*

PART A

	Dry matter of hay eaten	Metabolizable energy		Heat produced	Gain of energy
		Per pound dry matter	Total		
	Lbs.	Cals.	Cals.	Cals.	Cals.
Period 4.....	10. 21	935	9, 544	9, 812	-268
Period 3.....	6. 17	935	5, 768	8, 064	-2, 296
Difference.....	4. 04		3, 776	1, 748	2, 028
Difference per pound dry matter of hay.....			935	433	502

PART B

Period 4.....	10. 21	910	9, 291	9, 812	-521
Period 3.....	6. 17	960	5, 923	8, 064	-2, 141
Difference.....	4. 04		3, 368	1, 748	1, 620
Difference per pound dry matter of hay.....			834	433	401

PART C

Period 4.....	10. 21	960	9, 802	9, 812	-10
Period 3.....	6. 17	910	5, 615	8, 064	-2, 449
Difference.....	4. 04		4, 187	1, 748	2, 439
Difference per pound dry matter of hay.....			1, 036	433	603

In Table I, Part B, the metabolizable energy per pound of dry matter of the hay eaten is 910 Calories in period 4, and 960 Calories in period 3; the average being 935 Calories as in Table I, Part A. The apparent net-energy value of the hay per pound of dry matter is $1,620 \div 4.04 = 401$ Calories, or $834 - 433 = 401$ Calories, instead of 502 calories—a difference of 101 Calories, or 20 per cent. This difference in net-energy value is the direct result of the assumed difference of 50 Calories in the metabolizable energy per pound of dry matter of hay of the two periods compared, although the average metabolizable-energy value of the hay, and heat increment, remained unaltered. The metabolizable energy per pound of dry matter of the added hay is 834 Calories, which is considerably less than the value of the hay eaten in either period 3 or period 4.

³ For some sources of error in the determination of metabolizable energy see (17).

In Table I, Part C, the metabolizable energy per pound of dry matter of the hay eaten is 960 Calories in period 4, and 910 Calories in period 3; the average remaining the same, as in Table I, Parts A and B, namely, 935 Calories. The apparent net-energy value of the hay in this case is 603 Calories per pound of dry matter, as compared with 502 Calories in Table I, Part A, and 401 Calories in Table I, Part B, although the heat increment and the average metabolizable-energy values are the same in all three cases. The metabolizable energy per pound of dry matter of the added hay is in this case 1,036 Calories, which is considerably higher than the value of the hay eaten in either of the two periods.

The availability of the metabolizable energy of the hay, computed from the data which have just been given in Table I, Parts A, B, and C, would be, respectively, $502 \div 935 = 53.7$ per cent; $401 \div 834 = 48.1$ per cent, and $603 \div 1,036 = 58.2$ per cent.

It is obvious from the foregoing that the net-energy value computed by this method is affected not only by the difference in metabolizable-energy value of the feed between the two periods compared, but also by the direction of this difference; that is, by whether the determined metabolizable-energy value is greater in the heavier or the lighter of the two rations. Since the net-energy value is computed from the difference in gain between the periods, a relatively small error in the determination of the metabolizable-energy value, which affects the gain directly, may cause a large percentage error in the net-energy value, especially if the difference in gain between the two periods is small. Obviously, errors in heat production would tend to reduce the effect of errors in metabolizable energy, if these errors are in the same direction. If, however, they are in opposite directions, their effect on the gains and on the net-energy value computed by difference would be magnified. It is possible, therefore, to obtain absurd results by this method of computation.

This method was originally used by Armsby and Fries (1, 2, 3, 4) in the computation of the availability of the metabolizable energy, which means the net-energy value expressed as percentage of metabolizable energy, and may account to an appreciable extent for the discordant results contained therein.⁴

In their later publications (5, 6, 7, 9), Armsby and Fries expressed the net-energy values as per kilogram of dry matter of the feed, and have also recomputed the results of the early experiments on the same basis, using a simplified method of computation. This method consists of averaging the metabolizable-energy values from the several periods, and subtracting from this average the average heat increment of the feed, usually determined by a comparison of the greatest and least amounts of feed eaten. The net-energy value thus obtained is not affected by the factors previously discussed, as in computations by the first method. It is, however, an average value derived in some instances from quite discordant results, and as such does not show the variations in the individual periods.

The new modification described later makes possible the computation of a net-energy value of the feed for each period separately, and besides it has other advantages which will be illustrated in the following examples.

⁴ These publications cover experiments 174, 179, 186, 190, 200, and 207, the last three being included in Bulletin 128 of the Bureau of Animal Industry, United States Department of Agriculture.

Since in the following pages the comparison is between only the later method used by Armsby and Fries and the newly modified method of computation, the former will be referred to, for the sake of brevity, simply as the "current method" and the latter as the "new method." It should be borne in mind, however, that what is termed the "new method" of computation is merely a modification of the current method and is based on principles evolved and expounded by Armsby.

EXAMPLE 2, FROM EXPERIMENT 207

In order to make clear the derivation of the net-energy values by the current method and by the new method, a detailed explanation of the several computations involved in both is given in this example (Table II):

TABLE II.—Data for computation of net-energy values of timothy hay and grain mixture No. 1

Period No.	Animal	Average live weight	Dry matter eaten		Metabolizable energy		Heat production ^a	Gain of energy
			Timothy hay	Grain mixture No. 1	Per kgm. of dry matter	Total		
		Kgms.	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.
1-----	A	499	2. 9349	1. 9962	-----	12, 061	10, 171	+1, 890
2-----	A	519	2. 9487	4. 7590	-----	20, 553	14, 035	+6, 518
3-----	A	507	2. 9742	-----	2, 096	6, 235	7, 780	-1, 545
4-----	A	514	4. 8920	-----	2, 076	10, 157	9, 501	+656

^a The heat production used in this example, and in those following, has been corrected to a standard day of 12 hours standing and 12 hours lying according to the method of Fries and Kriss (10).

THE CURRENT METHOD

COMPUTATION OF THE HEAT-INCREMENT VALUES OF HAY AND OF GRAIN

Using the total observed heat production, corrected to the standard day of 12 hours standing and 12 hours lying as the starting point, the dry matter of the ration (kilograms) and the heat production of one period are compared with the dry matter and the heat production of the other periods. In case the comparison involves one feeding stuff, for instance hay alone, the difference in heat production is divided by the difference in dry matter. The result is the heat increment per kilogram of dry matter. In case the comparison involves a mixed ration of hay and grain, the difference in heat production due to the added hay is first computed by multiplying the latter by the heat-increment value previously determined from the hay rations. This is subtracted from the total difference in heat production, and the result is divided by the difference in grain to get the heat increment per kilogram of grain. Tables III and IV illustrate this computation, and give the heat-increment values of the timothy hay and of the grain mixture used in the experiment under consideration.

There is only one heat-increment value for the hay, namely, 897 Calories per kilogram of dry matter, which, of course, represents the average heat-increment value of the hay. For the grain there are three values—namely, 1,215 Calories, 1,394 Calories, and 1,319 Calories

per kilogram of dry matter. Of these, the one obtained by comparison of the greatest and least amounts of feed (periods 2 and 3)—namely, 1,319 calories—is considered as the average in the computation of the average net-energy value by the current method.

Attention is called to the fact that the heat-increment values as shown in Tables III and IV are different from those which have been published (5). This is the result of the use of revised figures for heat production as previously noted (see footnote to Table II).

TABLE III.—Computation of heat increment per kilogram of timothy hay

Period No.	Quantity of dry matter eaten	Heat production
	Kgms.	Cals.
Period 4.....	4. 8920	9, 501
Period 3.....	2. 9742	7, 780
Difference.....	1. 9178	1, 721
Difference per kilogram of dry matter.....		897

TABLE IV.—Computation of heat increment per kilogram of grain mixture

Period No.	Quantity of dry matter eaten		Heat production
	Hay	Grain	
	Kgms.	Kgms.	Cals.
Period 1.....	2. 9349	1. 9962	10, 171
Period 3.....	2. 9742		7, 780
Difference.....	— . 0393	1. 9962	2, 391
Difference due to hay.....			— 35
Difference due to the grain.....			2, 426
Difference per kilogram of grain.....			^a 1, 215
Period 2.....	2. 9487	4. 7590	14, 035
Period 1.....	2. 9349	1. 9962	10, 171
Difference.....	. 0138	2. 7628	3, 864
Difference due to hay.....			12
Difference due to 2.7628 kilograms of grain.....			3, 852
Difference per kilogram of grain.....			1, 394
Period 2.....	2. 9487	4. 7590	14, 035
Period 3.....	2. 9742		7, 780
Difference.....	— . 0255	4. 7590	6, 255
Difference due to hay.....			— 23
Difference due to the grain.....			6, 278
Difference per kilogram of grain.....			^b 1, 319

^a The same value will be obtained by comparing periods 1 and 4.
^b The same value will be obtained by comparing periods 2 and 4.

COMPUTATION OF THE AVERAGE METABOLIZABLE-ENERGY VALUE OF THE HAY AND OF THE GRAIN

The total metabolizable energy of each hay ration is divided by kilograms of dry matter. The results are metabolizable-energy values per kilogram of dry matter. These values for the timothy hay are (as in Table II) 2,096 Calories and 2,076 Calories for periods 3 and 4, respectively. The average of these—namely, 2,087 Calories—represents the average metabolizable-energy value of the hay used in the experiment.

The metabolizable-energy value of the grain is obtained by a calculation by difference from the hay and grain rations. The metabolizable-energy equivalent of the hay in the mixed ration is first computed by multiplying the average metabolizable-energy value of the hay, previously determined from the hay rations, by the dry matter of the hay of the mixed ration. This is subtracted from the total metabolizable energy of the ration. The remainder is the metabolizable energy of the grain. Dividing this by kilograms of dry matter of the grain gives the metabolizable-energy value of the grain per kilogram of dry matter. These values for the grain mixture are 2,975 Calories and 3,026 Calories per kilogram of dry matter in periods 1 and 2, respectively. The average of these—namely, 3,001 Calories—is the average metabolizable-energy value of the grain.

COMPUTATION OF THE NET-ENERGY VALUES OF THE HAY AND OF THE GRAIN

This computation consists of a simple subtraction of the average heat-increment value from the average metabolizable-energy value previously determined. The result is the average net-energy value of the feed. The average net-energy value of the timothy hay thus computed would therefore be 1,190 (2,087 – 897) Calories per kilogram of dry matter. The average net-energy value of the grain mixture would be 1,682 (3,001 – 1,319) Calories per kilogram of dry matter.

By dividing the average-net energy value per kilogram of dry matter of the feed by its average metabolizable-energy value per kilogram of dry matter, the average percentage utilization of the metabolizable energy may be obtained. According to this computation, the utilization of the metabolizable energy of the timothy hay would be 57.02 per cent ($1,190 \div 2,087$) and of the grain mixture 56.05 per cent ($1,681 \div 3,001$).

THE NEW METHOD

The new method involves the separate determination (1) of the net energy required for maintenance, (2) of the gain of energy by the animal, and (3) of the heat-increment value of the feed.

The heat production of an animal on feed represents a composite of the net energy required for maintenance, in the broad sense; that is, including voluntary muscular activities, and of the energy expenditure, or heat increment, due to the consumption of the feed. By means of the respiration calorimeter, the total heat production is directly measured. The heat-increment value of the feed is determined by a comparison of periods in which different amounts of feed are eaten, as described in the current method, and illustrated in Tables III and IV. This computation assumes that the net energy required for maintenance is the same in the periods which are compared, and that this assumed, although undetermined, maintenance value is canceled in subtracting the heat production of one period from that of another. To illustrate this, if H and H_1 represent, respectively, the heat production of two periods, h and h_1 , the heat increment due to the feed, and m the net energy required by the animal for maintenance in either period, then $H = m + h$, and $H_1 = m + h_1$. Subtracting the second expression from the first results in the equation $H - H_1 = h - h_1$.

The new method calls for a determination of the maintenance requirement (m) of net energy. This is obtained by subtracting the computed heat increment, due to the consumption of the feed, from the heat production of each period, in accordance with the principles outlined by Armsby (8, p. 282). The average of the several determinations is considered to represent the maintenance requirement of net energy. To this net energy for maintenance is added the gain of energy by the animal in each period, as obtained by subtracting the total heat production from the total metabolizable energy (Table II), and the result represents the total net energy of the ration, from which the net-energy value of the feed is computed. The specific directions for the computations, and the results obtained with the timothy hay and grain mixture No. 1, previously considered, are as follows:

COMPUTATION OF THE HEAT-INCREMENT VALUE OF THE FEED

The computation of the heat increment per kilogram of feed is exactly as in the current method. To avoid repetition, reference is made to the foregoing description of the current method, and to Tables III and IV, which illustrate this computation and give the heat-increment values. If there is more than one value for a feed, the average is used. This is a departure from the current method, in which the value obtained by comparison of only the two extreme rations was used. The reasons for this departure will be given later.

There is only one heat-increment value for the hay—namely, 897 Calories per kilogram of dry matter; and there are three values for the grain mixture—namely, 1,215 Calories, 1,394 Calories, and 1,319 Calories per kilogram of dry matter. The average of these is 1,309 Calories.

COMPUTATION OF THE NET ENERGY REQUIRED FOR MAINTENANCE

Multiply the average heat-increment value per kilogram of the feed by the kilograms of dry matter of each ration. The result is the total heat increment due to the feed. In case of a mixed ration of hay and grain, not fed in the same proportion in the different periods, multiply the kilograms of hay and of grain separately by their respective average heat-increment values, and add the results, to get the total heat increment due to the ration. Subtract the total heat increment from the heat production. The remainder is the net energy required for maintenance. Average the values thus obtained for the maintenance requirement of net energy.

TABLE V.—*Computation of maintenance requirement*

Period No.	Dry matter eaten		Average heat increment per kilogram of dry matter		Total heat increment of ration	Total heat production	Net energy for maintenance
	Timothy hay	Grain mixture	Hay	Grain			
	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.	Cals.
1.....	2.9349	1.9962	897	* 1,309	5,246	10,171	4,925
2.....	2.9487	4.7590	897	* 1,309	8,875	14,035	5,160
3.....	2.9742	897	2,668	7,780	} 5,113
4.....	4.8920	897	4,338	9,501	
Average.....	5,066

* Average of all values.

COMPUTATION OF THE GAINS OF ENERGY BY THE ANIMAL AND OF THE TOTAL NET ENERGY OF THE RATIONS

Subtract from the metabolizable energy of the ration the total heat production. The remainder is the net energy gained by the animal body. This gain may be negative on low rations. Add to the energy gained by the animal the average net energy required for maintenance. The sum represents the total net energy of the ration.

TABLE VI.—*Computation of the total net energy of the ration*

Period No.	Metabo- lizable energy	Heat pro- duction	Gain	Net en- ergy for mainte- nance	Total net energy of rations
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
1.....	12, 061	10, 171	+1, 890	5, 066	6, 956
2.....	20, 553	14, 035	+6, 518	5, 066	11, 584
3.....	6, 235	7, 780	-1, 545	5, 066	3, 521
4.....	10, 157	9, 501	+656	5, 066	5, 722

COMPUTATION OF THE NET ENERGY PER KILOGRAM OF DRY MATTER, AND OF THE PERCENTAGE UTILIZATION OF THE METABOLIZABLE ENERGY OF HAY

Divide the total net energy of the hay ration by the kilograms of dry matter eaten. The result is the net energy value per kilogram of dry matter. To get the percentage utilization of the metabolizable energy, divide the total net energy of the hay ration by the metabolizable energy of the ration and multiply the result by 100.

TABLE VII.—*Computation of the net-energy value of timothy hay*

Period No.	Dry mat- ter of hay eaten	Metabo- lizable energy of ration	Total net energy of ration	Net en- ergy per kilogram of dry matter	Utiliza- tion of the metabo- lizable energy
	<i>Kgms.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>
3.....	2. 9742	6, 235	3, 521	1, 184	56. 47
4.....	4. 8920	10, 157	5, 722	1, 170	56. 34
Average.....				1, 177	56. 40

COMPUTATION OF THE NET ENERGY PER KILOGRAM OF DRY MATTER, AND OF THE PERCENTAGE UTILIZATION OF THE METABOLIZABLE ENERGY OF GRAIN

Multiply the average net energy value of the hay previously determined from the hay rations (Table VII) by the dry matter of the hay in the mixed ration, to get the net energy equivalent of the hay. Subtract this from the total net energy of the ration. The remainder is the net energy of the grain. Divide the net energy of the grain by the kilograms of dry matter of grain eaten to get the net energy per kilogram of dry matter of grain. Multiply the average metabolizable-energy value of the hay by the dry matter of hay in the mixed ration, and subtract the result from the total metabolizable energy of the ration, to get the metabolizable energy of the grain. Divide the net energy of the grain by the metabolizable energy of the grain, and multiply the result by 100 to get the percentage utilization of the metabolizable energy of the grain.

TABLE VIII.—*Computation of net-energy value of grain mixture No. 1*

Period No.	Dry matter eaten		Total metabolizable energy of ration	Metabolizable energy equivalent of the hay	Metabolizable energy of the grain	Total net energy of ration	Net energy equivalent of the hay	Net energy of the grain	Net energy per kilogram dry matter of grain	Utilization of the metabolizable energy of the grain
	Hay	Grain								
	<i>Kgms.</i>	<i>Kgms.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>
1.....	2. 9349	1. 9962	12, 061	6, 125	5, 936	6, 956	3, 454	3, 502	1, 754	59. 00
2.....	2. 9487	4. 7590	20, 553	6, 154	14, 399	11, 584	3, 471	8, 113	1, 705	56. 34
Average.....									1, 730	57. 67

The net energy values computed by the new method for the timothy hay (Table VII) are 1,184 Calories and 1,170 Calories per kilogram of dry matter, in periods 3 and 4, respectively, the average being 1,177 Calories as compared with 1,190 Calories by the current method. For the grain mixture the net-energy values computed by the new method are 1,754 Calories and 1,705 Calories per kilogram of dry matter in periods 1 and 2, respectively, the average being 1,730 Calories as compared with 1,682 Calories by the current method. There is, likewise, a difference in the average percentage utilization of the metabolizable energy of the hay and grain as computed by the two methods. These differences are due to the differences in procedure. According to the new method, the average of the several determinations of the heat-increment values of the feed is made the basis for the computation of the maintenance requirement of the animal. This departure from the current method is made in consideration of the variations in the heat-increment values obtained by comparison of different periods and of the possible causes for these variations. From the nature of the computation and assumptions involved, any experimental error in the total heat production, or any possible influence of the plane of nutrition, or of difference in activity of the animal, on the net energy used for maintenance, would be reflected solely in the heat-increment value. The influence of such factors on the heat-increment value may be greatly exaggerated, if the difference in heat production and the difference in feed between the two periods compared are small. On this account, the consideration by Armsby and Fries of the value obtained by comparison of the greatest and least amounts of feed consumed as representing an average value in their computations of net-energy values is not without justification. However, since the maintenance requirement of the animal is a factor in the determination of the heat-increment values, the average of several determinations would afford a more accurate basis for the computation of the maintenance requirement during the several periods of an experiment than would a value computed only from the two rations of greatest and least amounts. In the particular example cited above the heat-increment value used for the hay was the same in both methods, there being only one value, while the difference between the average heat-increment value of the grain used in the new method and that used in the current method is only 10 Calories (1,319 and 1,309). This difference alone can not entirely account for the difference in the

average net-energy values of the hay and of the grain as obtained by the two methods. Apparently, the determination of the gains of energy by the animal in each period, and the use of a determined instead of an assumed average value for the net energy required for maintenance, are chiefly responsible for the difference in the average net-energy values as computed by the two methods. As regards differences in live weight in relation to the maintenance requirement of the animal, both methods assume that the variations in weight in one series of experiments are largely due to "fill," that is, to variations in the content of the digestive tract, rather than to any considerable change in the body proper, and that the actual maintenance requirement is not greatly affected.

In order that a full appreciation may be had of the differences between the two methods, in their application to different experiments, the following examples and discussion of results are presented.

EXAMPLE 3, FROM EXPERIMENT 211

TABLE IX.—Data for computation of net-energy values of mixed hay and hominy chop

Period No.	Animal	Average live weight	Dry matter eaten		Metabolizable energy		Heat production ^a	Gain of energy
			Mixed hay	Hominy chops	Per kilogram of dry matter	Total		
	Steer	Kgms.	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.
1.....	D	460	6.2042	-----	1,829	11,348	11,710	-362
2.....	D	432	1.7473	1.7637	-----	9,435	9,665	-230
3.....	D	470	3.9105	3.9488	-----	21,406	14,123	+7,283
4.....	D	455	3.4983	-----	1,990	6,958	9,302	-2,344
5.....	D	428	1.7864	-----	1,834	3,276	8,020	-4,744

^a Revised; see footnote to Table II.

TABLE X.—Heat increment per kilogram of dry matter ^a

Feeding stuff	Periods compared	Heat increment per kilogram
		Cals.
Mixed hay.....	1 and 4.....	890
Do.....	4 and 5.....	750
Do.....	1 and 5.....	835
Do.....	Average of all.....	825
Mixed hay and hominy chop.....	2 and 3.....	1,025
Computed for hominy chop.....	2 and 5.....	951
Do.....	3 and 4.....	1,134
Do.....	2 and 3.....	1,226

^a Computed from the revised figures for heat production.

The computation of the average net-energy values of the mixed hay, and of the hominy feed, according to the current method, from the data of Tables IX and X, would be as follows:

MIXED HAY.—Average metabolizable energy per kilogram of dry matter = 1,884 Calories. Heat increment per kilogram of dry matter obtained by comparison of the greatest and least amounts of feed (periods 1 and 5) = 835 Calories. Average net-energy value per kilogram of dry matter, $1,884 - 835 = 1.049$ Calories.

HOMINY FEED.—Average metabolizable energy per kilogram of dry matter, computed in a manner explained in example 2 = 3,522 Calories. Heat increment per kilogram of dry matter from periods 2 and 3 (greatest and least amounts of feed), computed in a manner illustrated in Table IV, example 2 = 1,226 Calories (Table X). Average net-energy value, $3,522 - 1.226 = 2.296$ Calories.

The computation of the maintenance requirement of the animal, and of the net-energy values of the mixed hay, and of the hominy feed, according to the new method, is as set forth in Tables XI, XII and XIII, respectively.

TABLE XI.—Computation of maintenance requirement

Period No.	Dry matter eaten		Average heat increment per kilogram dry matter		Total heat increment of ration	Total heat production	Net energy for maintenance
	Mixed hay	Mixed hay and hominy chop	Mixed hay	Mixed hay and hominy			
	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.	Cals.
1.....	6.2042		* 825		5,118	11,710	6,592
2.....		3.5110		1,025	3,599	9,665	6,067
3.....		7.8593		1,025	8,056	14,123	
4.....	3.4983		* 825		2,886	9,302	6,416
5.....	1.7864		* 825		1,474	8,020	6,546
Average.....							6,405

* Average of all.

TABLE XII.—Computation of net-energy values of mixed hay

Period No.	Dry matter of hay eaten	Net energy for maintenance	Gain	Total net energy of ration	Net energy per kilo. of dry matter
	Kgms.	Cals.	Cals.	Cals.	Cals.
1.....	6.2042	6,405	-362	6,043	974
4.....	3.4983	6,405	-2,348	4,061	1,161
5.....	1.7864	6,405	-4,744	1,661	930
Average.....					1,022

TABLE XIII.—Computation of net-energy values of hominy feed

Period No.	Dry matter eaten		Net energy for maintenance	Gain	Total net energy of ration	Net energy equivalent of the hay *	Net energy hominy feed	Net energy per kilo. of dry matter of hominy feed
	Mixed hay	Hominy feed						
	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.
2.....	1.7473	1.7637	6,405	-230	6,175	1,786	4,389	2,489
3.....	3.9105	3.9488	6,405	+7,283	13,688	3,997	9,691	2,454
Average.....								2,472

* Computed from the average value of table XI.

It will be noted that the computation of the net-energy values of the hominy feed by the new method did not involve, in this case, the computation of a heat-increment value for hominy feed alone. The fact that the hay and the hominy feed were fed in a definite proportion eliminated the necessity of such a computation, inasmuch as the heat increment of the mixed ration of the hay and hominy feed could be made use of directly in determining the value for maintenance in the periods in which the mixed ration was fed (periods 2 and 3, Table XI).

The net energy of the hominy feed computed by this method are 2,489 Calories, and 2,454 Calories per kilogram of dry matter, for periods 2 and 3, respectively, the average being 2,472 Calories, as compared with 2,296 Calories obtained by the current method. The net-energy values per kilogram of dry matter of the mixed hay are 974 Calories, 1,161 Calories, and 930 Calories, per kilogram of dry matter, for periods 1, 4, and 5, respectively, the average being 1,022 Calories instead of 1,049 Calories as by the current method. The considerable deviation of the net-energy value of the hay of period 4 from the values in the other two periods is rather to be expected, since an inspection of the heat-increment values (Table X) indicates that the heat production of period 4 is somewhat low for the ration as compared with the heat production of the other two hay periods, while Table IX shows the metabolizable-energy value in period 4 to be higher than the values in the other two periods, the effect therefore being to make the gains of period 4, and consequently the net-energy value of the hay of this period, relatively high. The individuality of the data is, apparently, preserved by the new computation of the net-energy values.

EXAMPLE 4, FROM EXPERIMENT 212

TABLE XIV.—Data for computation of net-energy values of alfalfa hay and alfalfa meal

Period No.	Animal	Average live weight	Dry matter eaten		Metabolizable energy		Heat production ^a	Gain of energy
			Alfalfa hay	Alfalfa meal	Per kilo-gram of dry matter	Total		
	Steer	Kgms.	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.
1.....	H	349	6. 6383	-----	2,009	13, 337	11, 134	+2, 203
2.....	H	349	-----	6. 6707	1,984	13, 238	10, 946	+2, 292
3.....	H	354	5. 3202	-----	2,002	10, 650	9, 843	+807
4.....	H	349	-----	5. 4078	1,971	10, 661	9, 767	+894
5.....	H	337	3. 0524	-----	2,133	6, 510	7, 394	—884
6.....	H	329	-----	3. 1549	2,088	6, 587	6, 868	—281

^a Revised; see footnote to Table II.

TABLE XV.—Heat increment per kilogram of dry matter ^a

Feeding stuff	Periods compared	Heat increment per kilo-gram	Feeding stuff	Periods compared	Heat increment per kilo-gram
		Calories			Calories
Alfalfa hay.....	1 and 3.....	980	Alfalfa meal.....	2 and 4.....	934
Do.....	3 and 5.....	1,080	-----do-----	4 and 6.....	1,287
Do.....	1 and 5.....	1,043	-----do-----	2 and 6.....	1,160
Do.....	Average of all.....	1,034	-----do-----	Average of all.....	1,127

^a Computed from the revised figures for heat production.

This experiment presents a striking example of what the writer believes is the advantage of computing net-energy values according to the new procedure.

According to the current method, the average net-energy value of the alfalfa hay, using the heat increment of the extreme periods (1 and 5, Table XV), would be $2,048 - 1,043 = 1,005$ Calories per kilogram of dry matter, and of the alfalfa meal $2,014 - 1,160$ (heat increment of 2 and 6) = 854 Calories. By using the average of all heat-increment values of the hay and meal, respectively, the average net-energy value would be $2,048 - 1,034 = 1,014$ Calories per kilogram of dry matter of alfalfa hay, and $2,014 - 1,127 = 887$ Calories per kilogram of dry matter of the alfalfa meal. Hence, in both cases, the average net-energy value of the meal is considerably lower than that of the hay, when computed by this method.

TABLE XVI.—*Computation of maintenance requirement*

Period No.	Dry matter eaten		Average heat increment per kilogram of dry matter ^a	Total heat increment of ration	Total heat production	Net energy for maintenance
	Alfalfa hay	Alfalfa meal				
	<i>Kgms.</i>	<i>Kgms.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
1.....	6.6383	6.6707	1,034	6,864	11,134	4,270
2.....			1,127	7,518	10,946	3,428
3.....	5.3202		1,034	5,501	9,843	4,342
4.....		5.4078	1,127	6,095	9,767	3,672
5.....	3.0524		1,034	3,156	7,394	4,238
6.....		3.1549	1,127	3,556	6,868	3,312
Average.....						3,877

^a Average of all values of hay and meal, respectively.

TABLE XVII.—*Computation of net-energy values of alfalfa hay and alfalfa meal*

Feeding stuff	Period No.	Dry matter eaten	Net energy for maintenance	Gain	Total net energy of ration	Net energy per kilogram of dry matter
		<i>Kgms.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
Alfalfa hay.....	1	6.6383	3,877	+2,200	6,080	916
Do.....	3	5.3203	3,877	+804	4,684	881
Do.....	5	3.0524	3,877	-883	2,993	981
Average.....						926
Alfalfa meal.....	2	6.6707	3,877	+2,269	6,169	925
Do.....	4	5.4078	3,877	+891	4,771	882
Do.....	6	3.1549	3,877	-286	3,596	1,140
Average.....						982

The net-energy values of the alfalfa hay for the different periods, as computed by the new method, show a fair agreement, the average being 926 Calories per kilogram of dry matter. The net-energy values of the alfalfa meal show a good agreement between periods 2 and 4, and a large deviation from the other values in period 6. Excepting this last period the values for the meal do not differ materially from those of the hay.

Turning to Table XVI, a considerable variation in the values for maintenance in the alternate periods may be noted, these values being lower in the periods in which alfalfa meal was fed. Such a variation can not be accounted for by differences in live weight, as these were very slight (Table XIV). Nor can it possibly be ascribed to the plane of nutrition, for the alfalfa hay rations and the alfalfa meal rations were practically the same. It is also unlikely that the activity of the animal would vary with such a regularity in the alternate periods. Tracing this back to the heat-increment values, in Table XV, it is to be noted that while the heat-increment value of alfalfa meal as obtained by comparison of periods 2 and 4 (934 Calories) is lower than the corresponding value for the hay (980 Calories), the heat-increment values of the meal involving period 6 are considerably higher than the corresponding values for the hay, making the average heat-increment value of the meal higher than that of the hay, and the values for maintenance lower. Whatever the ultimate cause may be, the indication is that when computed by the current method the divergent period 6 is responsible for making the average net-energy value of alfalfa meal considerably lower than that of alfalfa hay, which is hardly possible. It is apparent, therefore, that the new method of computation places the net-energy values on a more accurate plane.

EXAMPLE 5, FROM EXPERIMENT 186

TABLE XVIII.—Data for computation of net-energy values of red clover hay

Period No.	Animal	Average live weight	Dry matter eaten	Metabolizable energy		Heat production ^a	Gain of energy
				Per kilogram of dry matter	Total		
	Steer	Kgms.	Kgms.	Cals.	Cals.	Cals.	Cals.
1a.....	I	572	2.9333	2,019	5,922	10,597	-4,675
1b.....			2.9333	2,019	5,922	11,321	-5,398
2a.....			5.0253	2,129	10,690	11,268	-578
2b.....			5.0253	2,129	10,690	11,113	-423
3a.....			4.1391	2,082	8,614	10,605	-1,991
3b.....			4.1391	2,082	8,614	10,677	-2,062

^a Revised, see footnote to Table II.

TABLE XIX.—Heat increment per kilogram of dry matter of red clover hay ^a

Series	Periods compared	Heat increment per kilogram	Series	Periods compared	Heat increment per kilogram
		Cals.			Cals.
a.....	1a and 3a.....	7	b.....	1b and 3b.....	-534
a.....	2a and 3a.....	748	b.....	2b and 3b.....	492
a.....	1a and 2a.....	321	b.....	1b and 2b.....	-99

^a Computed from the revised figures for heat production.

Experiment 186 has been considered by Armsby and Fries as an unsatisfactory experiment, as judged by the abnormally low and divergent heat-increment values. The computation of these values from the revised figures for heat production (Table XIX) reveals this

experiment as even more unsatisfactory. Without going into the causes for these results here, this experiment may serve to show how it is possible, by the new method, using a previously determined maintenance value for the animal, to reveal the general character as to accuracy of the experimental data.

The average net-energy value per kilogram of dry matter of the red clover hay, computed from the data of series *a* according to the current method, would be $2,076 - 321 = 1,755$ Calories, and computed from the data of series *b*, impossible values, as indicated in the table.

Experiment 179 was a satisfactory experiment with the same steer, conducted one year before experiment 186, with the same kind of feed as in experiment 186 (red clover hay) in two of the four periods. The maintenance requirement of the animal, computed from the revised figures for heat production, was found to be 6,618 Calories of net energy. The average live weight of the animal during that experiment was 528 kilograms, as compared with 572 kilograms during experiment 186, differing by only 44 kilograms. The maintenance requirement of the steer during experiment 186 may therefore be computed with a fair degree of accuracy in proportion to the two-third power of the live weights as follows:

$$6,618 \times \left(\frac{572}{528}\right)^{2/3} = 6,981 \text{ Calories.}$$

By the use of this value for maintenance, approximate net-energy values of the red clover hay in experiment 186 may be computed as shown in Table XX.

TABLE XX.—*Computation of net-energy values of red clover hay*

Period No.	Dry matter eaten	Net energy for maintenance	Gain	Total net energy of ration	Net energy per kilogram of dry matter
	<i>Kgms.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
1a.....	2.9333	6,981	−4,675	2,306	786
1b.....	2.9333	6,981	−5,398	1,583	540
2a.....	5.0253	6,981	−578	6,403	1,274
2b.....	5.0253	6,981	−423	6,558	1,305
3a.....	4.1391	6,981	−1,991	4,990	1,206
3b.....	4.1391	6,981	−2,062	4,919	1,188

The net-energy values as computed in Table XX are in fair agreement for periods 2 and 3 of each series, while those of period 1 are abnormally low. This points to an abnormally high heat production in period 1 of each series, as the variation in the metabolizable-energy values in the different periods is only slight (Table XVIII). In other words, the results given in Table XX indicate that period 1 of each series was mainly responsible for rendering the whole experiment worthless when computed by the current method. The new computation indicates that the results of periods 2 and 3 of both series *a* and *b* are fairly accurate. The advantage of such a computation is obvious.

SUMMARY

By a critical analysis of the methods of this institute for the computation of the net-energy values of feeds, from results of respiration calorimeter experiments, previously unsuspected defects have been revealed.

CEREAL INVESTIGATIONS.

These defects have seriously affected the general character, as to accuracy, of the existing evidence in the field of net-energy determinations.

Two procedures have been used in computing net-energy values, and have been widely quoted. Both are correct in principle, but tend to magnify experimental errors.

In the earlier method the net-energy value of a feed was determined by comparison of the gains in energy yielded by rations of different amounts. The feed represented in the computation was only the difference between the two rations, the relatively extensive maintenance requirement of net energy not being directly involved, the effect, therefore, being to relate the entire error of the determination to the small amount of feed represented by the gain.

The later method is an improvement over the earlier one in that the above-mentioned exaggeration of errors is eliminated. It tends, however, to obscure the individuality of the data, and to exaggerate the effects of assumptions necessarily involved.

A revised method of computation of net-energy values is presented which tends to eliminate the exaggeration of errors and to preserve the individuality of the data, and makes possible the computation of a net-energy value of a feed for each of a series of periods, instead of giving only one average value representing results of two or more periods, as accomplished by the earlier methods. It thus also provides an improved basis for judgment as to the consistent character of an experiment as a whole.

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IDENTITY OF THE MEALYBUG DESCRIBED AS DACTYLOPIUS CALCEOLARIAE MASKELL¹

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INTRODUCTION

The increasing importance of the elongate gray mealybug found in many parts of the world on sugar cane, both as a pest and as a subject for study as a possible carrier of the sugar-cane mosaic disease, has made it highly desirable to verify or correct the scientific name by which the species is recognized in technical literature. The specific name *calceolariae*, first applied to a mealybug more than 50 years ago, has in recent years been used very frequently in published references to this sugar-cane mealybug, although it has been recognized for some time that there was a legitimate question as to the correctness of the application.

In the course of preliminary studies on the large Maskell collection of scale insects, very generously loaned to the United States Bureau of Entomology by the New Zealand Department of Agriculture, a certain amount of material bearing on this subject was obtained, but nothing from which definite conclusions could be established. A recent appeal for additional specimens, made to J. G. Meyers, of the Biological Laboratory of the New Zealand Department of Agriculture, and transmitted by him to Gilbert Archey, of the Canterbury Museum, Christchurch, New Zealand, has resulted in the receipt from the latter of a few more specimens, some of which are mounted and accompanied by data indicating without question that they were before Maskell at the time he prepared his original description.

From the material thus accumulated it has been possible, after extended and careful study, to work out the identity of the species represented, if not completely, at least to an extent sufficient to meet the practical needs of the situation. This discussion attempts to restrict properly the use of the name *calceolariae*, and to segregate and correctly designate the different species which have been known by this name.²

HISTORY OF MASKELL'S USE OF NAME

The species was first described by Maskell in 1879 (9, p. 218-219),³ who then stated that "this insect is effecting great destruction in the public gardens in Christchurch amongst the *Calceolarias* and upon several native plants such as *Traversia*, *Cassina*, etc." In the original description the color is given as pink covered with white meal, and the shape is indicated as oval in the accompanying figure. The body is said to be very oily.

¹ Received for publication October 25, 1924; issued October, 1925.

² The drawings illustrating the structural characteristics of the species described here have been prepared under the writer's direction, those for *boninsis* by Emily Morrison, and the others by Leola J. Kruger.

³ Reference is made by number (italic) to "Literature cited," p. 499.

Maskell's second reference to the species was in 1884 (10, p. 138-139), when the insect was reported from Stewart Island, southern New Zealand, on *Phormium* and on a grass (*Danthonia*). The length of the *Danthonia* specimens, more than $\frac{1}{4}$ inch, was noted, and the species was considered to be indigenous.

The third reference was made in 1887 (11, p. 100), when the species was described again and reported as occurring on *Calceolaria* and *Phormium tenax* at Christchurch, and on *Danthonia* from Stewart Island. A colored figure of the species as it occurs on *Phormium* was given.

The species was next mentioned in 1890 (12, p. 149-150), when Maskell stated that examples received on sugar cane from Fiji were, to him, indistinguishable from *calceolariae*.

Another reference to the species was made in 1894 (13, p. 89), when specimens from Napier (New Zealand) on *Cordyline australis* were discussed. The same note suggested that a mealybug from northern Mexico is this species, since a drawing of the foot of this last exactly corresponds to that of *calceolariae* and since he (Maskell) has recorded this species from sugar cane.

In a "Synoptical list of the Coccidae reported from Australasia and the Pacific Islands up to December, 1894" (14, p. 24-25), Maskell recorded this species from New Zealand on *Calceolaria* sp., *Cordyline australis*, *Danthonia cunninghamii*, and *Phormium tenax*, and from Fiji on *Saccharum officinarum*.

Maskell's final reference to the species came in 1897 (15, p. 322) when he described as new a variety, *minor*, from Mauritius on roots of "onion grass."

MASKELL MATERIAL AVAILABLE FOR EXAMINATION

The following is a list of Maskell specimens, identified as this species, which have been available for examination:

(1) Original or type slides, "from Traversia, old female, June 1878"; "from Traversia, old females, June 1878"; "from Traversia, 2 young insects, June 1878"; "from *Calceolaria*, Female, 2nd stage, June 1878."

(2) Some slide mounts from specimens in formalin, received with the preceding slides from the Canterbury Museum, Christchurch, New Zealand, and labeled simply "*Dactylopius calceolariae*."

(3) Four slides from the Maskell collection, one each "From *Danthonia* (grass); (Stewart's Island), Adult female, Sept. 1880"; "adult females, 1886"; "larva, 1893"; "var. *minor*, adult female, 1896."

(4) Some slides of various stages prepared from the unmounted Maskell collection material.

(5) Two slides of different stages of var. *minor* prepared from the unmounted Maskell collection material.

STATUS OF THE MATERIAL LISTED

(1) It is obvious that only the first four slides listed can be positively considered as having been examined by Maskell at the time he described the species, and these, therefore, include the type.

(2) Of the four original slides, one, that from *Calceolaria*, bears only a poorly preserved second-stage female. This species is un-

doubtedly a close relative of *Pseudococcus citri* (Risso), differing from specimens of the same stage of that species, so far as the writer can determine, only in the possession of relatively large, short tubular ducts scattered adjacent to the cerarii, at least in the abdominal region. Since it is not possible to identify this definitely, it has been excluded from further consideration as part of the type material of the species.

(3) The three Maskell slides from Traversia therefore become the types of *Dactylopius calceolariae* of Maskell and the redescription given below under the name *Pseudococcus calceolariae* (Maskell) has been drawn up from the three adult females, all imperfect, present on these slides.

(4) The species mentioned by Maskell in 1883 as having been found on *Danthonia* at Stewart Island is distinct from both of the species already mentioned, and since it has not been recognized as identical with any of the described species in the group, it has been described here as new under the name *Trionymus danthoniae*.

(5) No specimens definitely known to have formed a part of the material on which the 1883 record on *Phormium* was based are available, so the actual status of this record is not determinable. However, in view of the widespread occurrence on *Phormium* of the species discussed in the next paragraph, it seems a reasonable assumption that the portion of Maskell's 1883 record referring to "*calceolariae* on *Phormium*" actually refers to the following species.

(6) Maskell's 1887 record on *Phormium tenax* from Christchurch is considered to be plainly represented by the single slide bearing two adult females, dated 1886. The specimens on this slide are specifically identical with some, without data other than name, mounted from the Maskell general collection, and with specimens on *Phormium tenax* from Berkeley, Calif., and despite certain apparent discrepancies, agree well with the description of *Pseudococcus diminutus* published by Leonardi. All the published records of *P. calceolariae* Mask. from the host *Phormium tenax* can therefore, in all probability, be referred to Leonardi's species *diminutus*.

(7) The specimens on sugar cane from Fiji, the record of which was published by Maskell in 1890, are not definitely represented by any of the specimens available for examination, and it is therefore impossible to establish with certainty the identity of the species to which this reference applies. In view of the wide distribution of the elongate gray mealybug of sugar cane, which has almost always been designated by the name *calceolariae* by other writers, it is a reasonable, although at present unverifiable, assumption that Maskell's specimens on sugar cane from Fiji were this species, that is, the *calceolariae* on sugar cane of authors. To this species the writer, in making identifications, has applied the name *boninsis* Kuwana, since the description given by Kuwana, so far as it extends, entirely coincides with specimens of this elongate gray sugar-cane mealybug already reported from so many parts of the world that its eventual discovery may be expected practically everywhere that sugar cane is grown extensively.

(8) The Maskell slide of a larva dated 1893 evidently is the species reported from Napier on *Cordyline australis*. None of the other specimens available can be definitely associated with this record, and the identity of the species can not be determined with

certainty from the larva. However, since the host is included in the same plant family as *Phormium*, since the general locality of the collection is identical with that of the species from *Phormium*, and since the larval characters, so far as they can be observed, do not contradict the conclusion, it is reasonable to assume that the species mentioned in this record is identical with that occurring on *Phormium*, and it is tentatively given such an association.

(9) After an examination of the type specimens, Maskell's *Pseudococcus calceolariae*, variety *minor*, has been placed as a synonym of *Pseudococcus citri* (Risso).

(10) Certain specimens, obtained from the unmounted portions of the Maskell and Canterbury Museum collections of "*calceolariae*," are not identical with any of the species already discussed and can not be positively associated with any of the Maskell records not represented by properly identified specimens. One of these species is characterized here, despite its lack of definite host or distribution records, in the hope that such description will expedite the discovery of its habitat and host relationships. The condition of the specimens of the other species is such as to preclude the possibility of accurate description, although it can probably be recognized by direct comparison if ever rediscovered.

DESCRIPTION OF SPECIES

Genus PSEUDOCOCCUS Westwood

Pseudococcus ambiguus, new species (fig. 1)

ADULT FEMALE.—(Described from a single mounted specimen.) Length 4 mm., width 2 mm., uniformly elongate oval; antennae broken, legs broken except coxae, the hind pair with a few large pores; beak small, elongate conical, length 153 μ , width 107 μ , somewhat obscurely 2-segmented; with the cephalic pair of cerarii present, each composed of 3 spines surrounded by a cluster of triangular pores without definitely associated setae; in addition with 4 more or less distinctly recognizable cerarii on each half of the posterior abdominal segments, the apical and preapical each with a densely crowded cluster of triangular pores, the remainder with scattered clusters, apical cerarii each with 4 long spines and 5 accessory setae, preapical with 6 to 8 spines of unequal size and 2 to 3 accessory setae, next two with 3 small spines and a few pores but no accessory setae, last (anterior) with 2 slender spines and a few pores only; remaining cerarii not definitely developed, but their location sometimes hinted at by presence of 1 or 2 well separated, slender spines accompanied by a slightly closer grouping of the adjacent triangular pores; anal lobes only slightly produced, no ventral chitinated thickening evident, apical setae broken, but, from diameter of base, apparently somewhat longer than anal ring setae; anal ring not unusual, with the usual inner and outer pore bands and 6 setae, the longest of these about 180 μ , pores of the usual types, the triangular widely scattered over both the dorsal and the ventral surfaces, the multilocular disk pores apparently limited to two small clusters, one anterior, the other posterior to the genital opening, plus a few in the middle of the segments just anterior to these; small tubular ducts present but rare, apparently confined to the posterior ventral abdominal area; body setae rather small and not conspicuous, the ventral averaging distinctly longer than the dorsal, more slender and varying considerably in size; ventral cicatrix moderately large, transverse, irregular in shape.

PREADULT FEMALE.—(Based on a single mounted specimen.) In general resembling the adult, but with somewhat smaller size or fewer numbers of various structures, and with multilocular disk pores wanting.

The holotype of this species was mounted from a pinned specimen included among the specimens of *Pseudococcus calceolariae* in the Maskell collection. As has already been demonstrated in this paper, the species is not *calceolariae*, and it is likewise possible to

reach the definite conclusion that it is not *Pseudococcus glaucus*, as originally described by Maskell, from the Maskell material of which the preadult female was obtained. The writer has not been successful in associating these specimens with any of the species described by Maskell or others, and although reluctant to describe it from the limited material available for study, he believes that it can be readily recognized from the description whenever it may be found in the future, owing to the apparently unusual and distinctive cerarian characters, and that its association with the species complex discussed in this paper makes its characterization practically necessary.

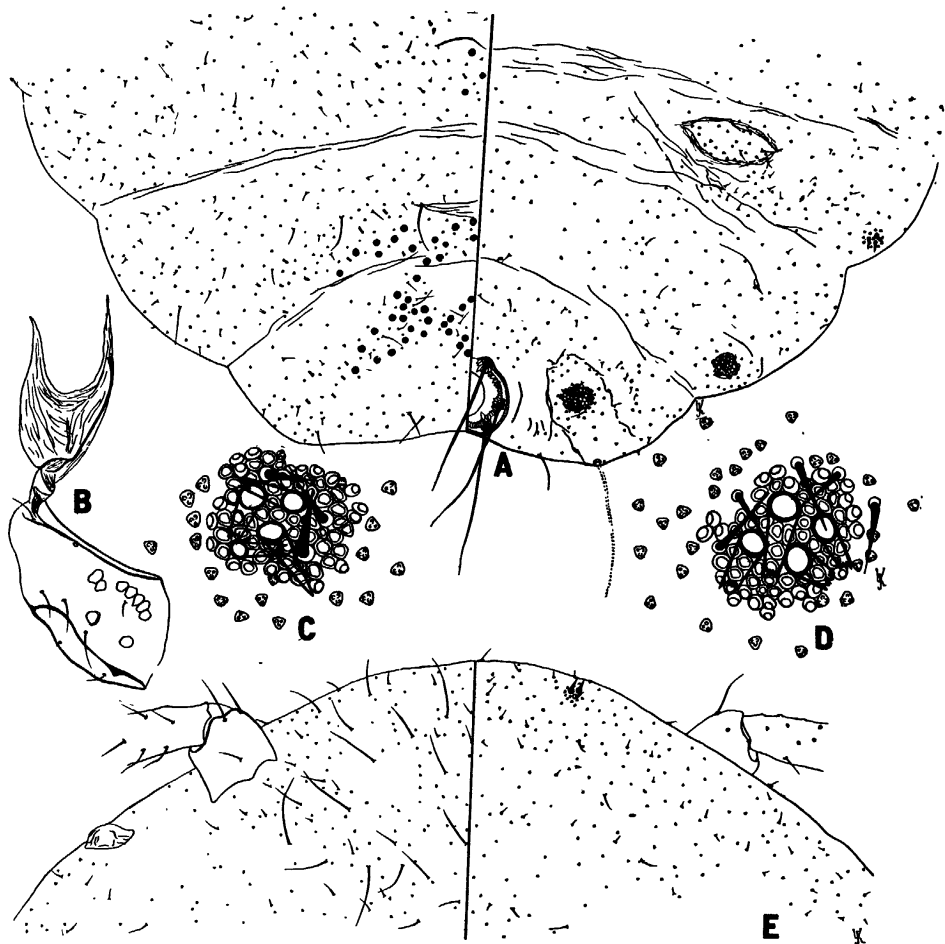


FIG. 1.—*Pseudococcus ambiguus*, adult female: A, apex of abdomen, $\times 96$; B, posterior coxa, $\times 96$; C, pre-apical cerarius, $\times 424$; D, apical cerarius, $\times 424$; E, apex of head, $\times 96$

Although no definite distribution or host data are available, it is assumably a New Zealand species.

The types are in the United States National collection of Coccidae.

PSEUDOCOCCUS BONINSIS KUWANA (figs. 2, 3)

ADULT FEMALE.—External appearance well described by Fullaway (3), and others, and not treated here; size of body, as mounted, varying considerably; well-developed individuals about 4.5 mm. long by 2.5 mm. wide; derm entirely membranous; antennae normally 8-segmented, the average lengths of the segments of several individuals in microns as follows: I, 67; II, 68; III, 43; IV, 31.5; V, 41; VI, 34; VII, 41; VIII, 98; legs not unusual, hind coxae with a varying number of tiny and often rather indistinct pores on basal portion, hind femora with some

large, faint areolations, not always evident; tibia about twice length of tarsus, about two and one-half times in hind pair, claw without denticle, both pairs of digitules long, slender, knobbed, but those of claw stouter; beak elongate conical, fairly evidently 2-segmented; series of cerarii incomplete, one pair on head and the 5 or 6 posterior abdominal pairs only present, each of those on head composed of 2 spines, a few triangular pores and 1 or 2 accessory setae, rarely with one or both of the spines elongated and setalike, still more rarely with 3 spines, the apical abdominal pair each large, with 2 spines, a loose cluster of pores and several accessory setae, not underlaid by any definite chitinous thickening, the remaining pairs with the spines progressively smaller anteriorly, each with 2 spines, a few triangular pores and one or more accessory setae, spines in each of sixth or anterior developed abdominal cerarii most often elongate, setalike; anal lobes hardly protruding, apical seta fairly large, about $100\ \mu$ long when uninjured, longest anal ring seta about $64\ \mu$; with the usual types of pores and ducts, triangular disk pores well distributed over body both dorsally and ventrally, apparently scattered or in very poorly defined broad segmental bands, multilocular disk pores confined to the ventral abdominal region, in a large cluster



FIG. 2.—*Pseudococcus boninsis* adult female. Apex of abdomen, $\times 185$

around the genital opening, and in two transverse rows or narrow bands on the two ventral segments anterior to the genital clusters, in no case, so far as has been determined, occurring in the head, thoracic, or anterior abdominal region, and none of the bands or clusters attaining the body margin at any point; only medium or rather small, short tubular ducts present, these, in the abdominal area, most abundant along the body margin adjacent to the cerarii, but present to some extent in the median area both dorsally and ventrally; body setae, both dorsally and ventrally, comparatively long and slender, showing considerable variation in length, those of ventral area actually and relatively longer; anal ring not unusual, with inner and outer single pore bands and 6 setae; ventral cicatrix of medium size, roughly quadrate, with rounded corners.

PREADULT FEMALE.—In general, rather closely resembling the adult except for smaller size and extent of development of the different structures; multilocular disk pores wanting.

This species has been redescribed from a considerable series of specimens, all from sugar cane, from Argentina, Bermuda, Brazil,

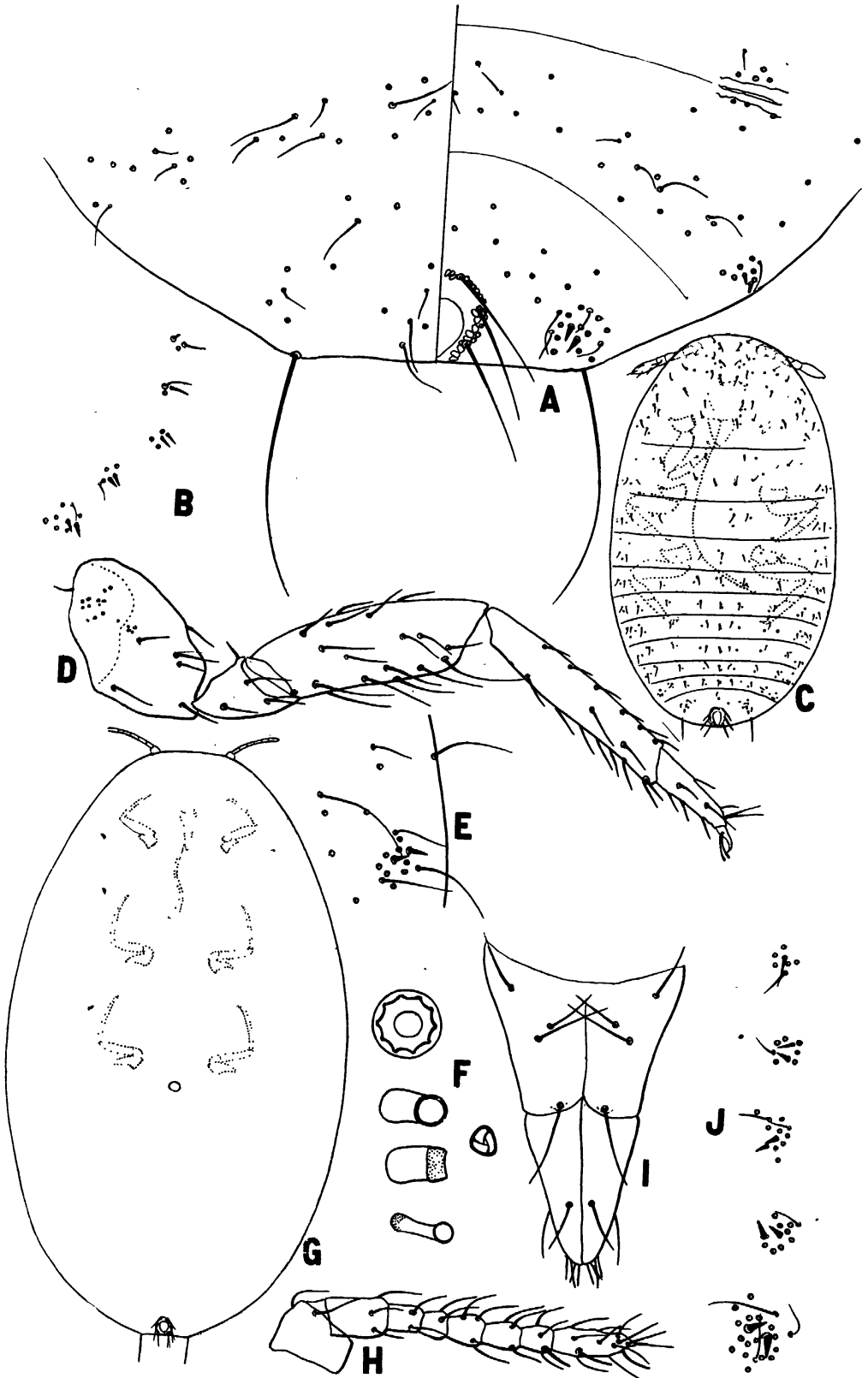


FIG. 3.—*Pseudococcus boninsis*: A, preadult female, apex of abdomen, $\times 230$; B, same, series of abdominal cerarii, $\times 230$; C, larva, outline, optical section, $\times 60$; D, adult female, posterior leg, $\times 115$; E, same, cerarius on head, $\times 230$; F, same, types of pores on body, $\times 1275$; G, same, outline of body, optical section, $\times 17.5$; H, same, antenna, $\times 115$; I, same, beak, $\times 230$; J, same, series of abdominal cerarii, $\times 230$

Canal Zone, District of Columbia (greenhouse), Dominican Republic, Florida, Georgia, Hawaiian Islands, Japan, Louisiana, and Panama. It has, unfortunately, not been possible to obtain specimens from the type material to prove absolutely the correctness of the association of these specimens with the species described by Kuwana on sugar cane from the Bonin Islands.

The only exceptions to the use of the name "*calceolariae*" for the gray sugar-cane mealybug in recent literature appear to be those found in the references of Kotinsky (6) and Ehrhorn (1; 2, p. 237). The first writer evidently confused and reversed the application of specific names to the two species *boninsis* and *sacchari* (Cockerell). The second author, on the basis of a comparison of specimens by E. E. Green, assigns the species *calceolariae* on sugar cane of authors to the species *saccharifolii* of Green. If one is to accept Green's original description as accurate, there is no possibility that the form here designated as *boninsis* can be identical with *saccharifolii*, since Green (5, p. 23-24) states very definitely that there is a group of about 8 stout pointed spines on each lobe of the apical abdominal segment and that the other segments, including both abdominal and thoracic, bear from 4 to 6 similar spines on processes on each margin. This description of the cerarian spines and that given of the distribution of the multilocular disk pores is so precise and so very different from the condition in *boninsis* (*calceolariae* on sugar cane of authors) that there would seem to be no possibility whatever of confusing the two species. Should the description prove to be seriously erroneous, and the type specimens actually identical with the species here designated as *boninsis*, Green's name will, from the dates of publication of the species, take precedence, since *boninsis* was described by Kuwana (7, p. 161-162) in 1909, more than one year later than the publication date of Green's species.

The latest contribution to this subject is that made from Hawaii by Fullaway (3, p. 312-314), where the name *Trionymus calceolariae* is used for the species under discussion. None of the specimens examined shows clearly the heavy ventral, chitinized anal lobe thickening emphasized by Fullaway in his drawing, although in many specimens the structure is vaguely suggested.

PSEUDOCOCCUS CALCEOLARIAE MASKELL (fig. 4)

ADULT FEMALE.—(Described from slide mounts only. Nothing available regarding external appearance, color, or secretion. See Maskell description for this.) Length as mounted averaging about 3 mm., width a little more than 1.5 mm., derm clearing completely except for appendages, and a tendency towards a thickening underlying each anal cerarius; antennae normally 8-segmented; measurements in microns about as follows: I, about 64; II, 40 to 78, average about 65; III, 57 to 93, average about 72; IV, 33 to 57, average about 46; V, 36 to 64, average about 48; VI, 36 to 43, average about 40; VII, 43 to 50, average about 47; VIII, 93 to 117, average about 107; legs not unusual, hind pair, so far as can be determined, without pores, except possibly a few large faint ones, femur slightly longer than tibia, claw stout, somewhat curved, without denticle, claw digitules rather stout, swollen at apices, exceeding claw, tarsal digitule slender, only slightly knobbed, likewise very slightly exceeding claw; beak elongate conical, appearing distinctly 2-segmented; with 17 pairs of cerarii made up of spines, triangular pores, and accessory setae, all, except the two or three anterior pairs, with 2 spines in each, these with 3, the spines of the posterior cerarii much larger than the others, intermediate cerarii with, on the average, about 10 pores and 2 accessory setae, but these numbers varying, each posterior cerarius with as many as 10 large accessory setae and around 50 triangular pores, these scattered, each posterior cerarius underlain by a chitinous thickening, fairly distinct in stained mounts; apical seta about 200 μ long, without any definite ventral thickening accompanying it; anal ring typical for the genus,

with inner and outer pore bands and 6 setae averaging about $196\ \mu$ long; body with the usual type of pores, the small triangular sort occurring both dorsally and ventrally in loose and indistinct transverse bands in the abdominal region, apparently indiscriminately scattered anteriorly, multilocular disk pores present ventrally in 5 transverse segmental rows in the abdominal region; large tubular ducts apparently confined to 1 opposite or nearly opposite and dorsal of each cerarius, and 1 or perhaps sometimes more on the median line anterior to the anal ring, small tubular ducts present in a loose cluster beneath each of the posterior 5 or 6 pairs of abdominal cerarii, the size of the clusters diminishing anteriorly, number and arrangement of these pores apparently showing con-

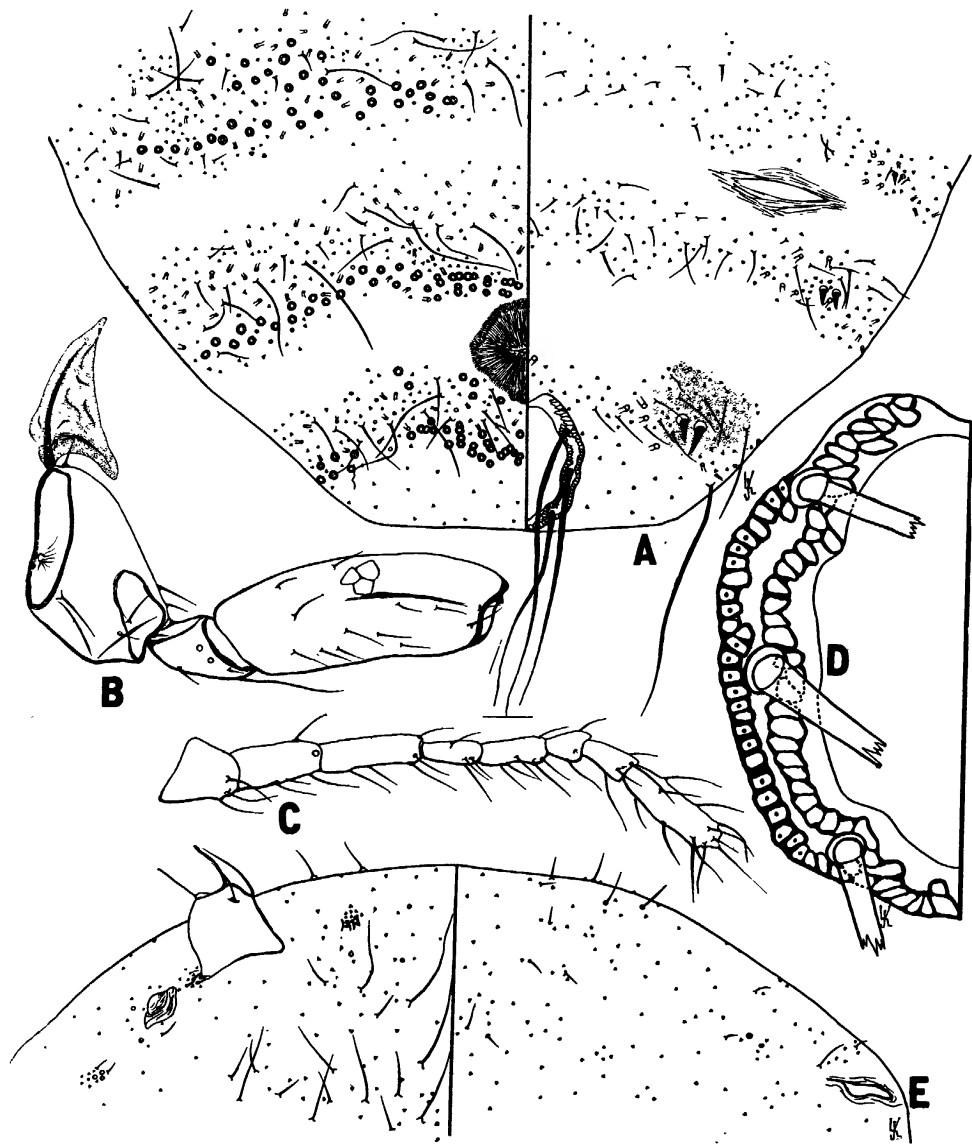


FIG. 4.—*Pseudococcus calceolariae*, adult female: A, apex of abdomen, $\times 120$; B, portion of leg, $\times 120$; C, antenna, $\times 120$; D, anal ring, $\times 530$; E, apex of head, $\times 120$

siderable variation, and in indefinite transverse ventral rows accompanying the large disk pores; dorsal setae small, in scattered transverse bands, ventral setae varying, but mostly much longer and relatively more slender, quite conspicuous, particularly anterior to the mouth parts; with a single, unusually large, quadrate ventral cicatrix.

This species has been redescribed from the three imperfect adult females from the original Maskell slide mounts having the following data: "from *Traversia*, June 1878."

This insect should be added to the group of species including *maritimus* (Ehrhorn), *adonidum* (Linnaeus) and *comstocki* (Kuwana). It resembles the last more closely than any other mealybug that the writer is familiar with, having the general organization of the pores and ducts and the posterior cerarii almost identical, but differing in apparently lacking a ventral chitinized thickening, and, on the head, in having the dorsal body setae shorter and more conspicuously

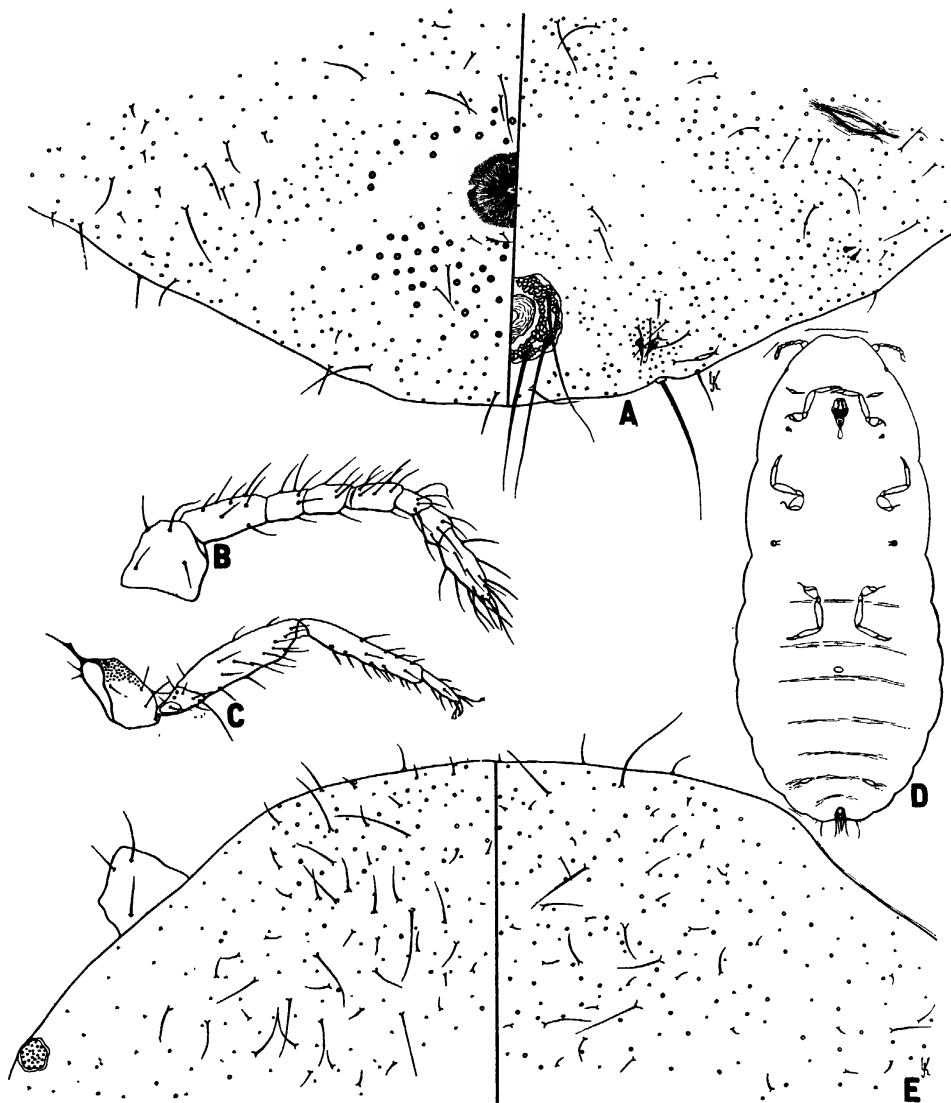


FIG. 5.—*Trionymus danthoniae*, adult female: A, apex of abdomen, $\times 96$; B, antenna, $\times 96$; C, posterior leg, $\times 96$; D, outline of body, optical section, $\times 10$; E, apex of head, $\times 96$

contrasted with the ventral in size, and finally in having fewer multilocular disk pores and ventral tubular ducts than are present in *comstocki*.

Genus *TRIONYMUS* Berg

Trionymus danthoniae, new species (fig. 5)

ADULT FEMALE.—(See Maskell paper (10, p. 138–139) for all available information regarding external appearance; described here from slide mount only.) Length 5.75 mm., width 2.5 mm., nearly parallel-sided with ends of body rounded, segmental constrictions more or less evident; antennae normally 8-segmented, average length of the segments in microns about as follows: I, 80; II, 89; III, 52;

IV, 45; V, 57; VI, 34; VII, 43; VIII, 86; the second segment thus the longest; legs not unusual, hind coxae with pores near base, at least, claw rather stout, somewhat curved, claw digitules stouter than those of tarsus, both pairs exceeding the tip of the claw, tibia and femur about equal in length, tibia about three times length of tarsus; beak stout conical, about as broad at base as length; with only the two posterior pairs of cerarii with the spines developed, these with 2 stout conical spines accompanied by several triangular pores in each, and with a loose cluster of 4 to 5 setae outside the spines; location of some additional cerarii indicated by presence of 1 or 2 larger setae at appropriate place on body margin, but no other definite cerarii present; anal lobes, as such, not indicated, apical seta large, about $196\ \mu$ long, without ventral chitinous thickening but with a ventral subapical seta about $90\ \mu$ long; body with small triangular disk pores of the normal type, occurring both dorsally and ventrally in indistinct transverse bands of widely scattered pores, with circular multilocular disk pores in indefinite transverse bands of scattered pores in the posterior abdominal region, with a few pores distributed apparently indiscriminately over the midventral area as far anteriorly as around the base of the mouth parts, and with numerous short tubular ducts distributed much in the same fashion as the triangular pores, but most numerous and conspicuous along the body margin; body setae scattered, varying considerably in size, with large setae both dorsally and ventrally, more conspicuous on the head than elsewhere; anal ring not unusual, with the usual inner and outer bands of pores and normally 6 setae, the longest about $183\ \mu$; with a single, small, transversely oval, ventral cicatrix placed on the median line well back of the posterior legs.

This species has been described from two specimens from the Maskell collection, one, the holotype, one of Maskell's slides bearing the notation "*Dactylopius calceolariae*. From Danthonia (grass) (Stewart's Island). Adult. Sept. 1880, W. M. M.," the other mounted from the bottle of miscellaneous specimens labeled "*Dactylopius calceolariae*," but without further data, received from the Canterbury Museum.

The holotype has been returned to New Zealand, and the paratype has been retained in the United States National collection of Coccidae.

TRIONYMUS DIMINUTUS (LEONARDI) (fig. 6)

ADULT FEMALE.—External appearance extensively described and figured by Maskell (11, p. 100) (as *calceolariae*) and by Leonardi (8); size apparently variable, maximum length as mounted 5 mm., maximum width 2.5 mm., but most examples smaller than this; antennae normally 8-segmented, average lengths of segments in microns about as follows: I, 72; II, 76; III, 50; IV, 41; V, 49; VI, 34; VII, 45; VIII, 96; legs not unusual, hind coxae with pores near base, lengths of this leg in microns: Coxa 215, trochanter 125, femur 243, tibia 268, tarsus 115, claw 35, without denticle, claw digitules thickened somewhat, tarsal slender, both expanded at apices and exceeding tip of claw; beak short conical, about $125\ \mu$ long and $118\ \mu$ wide at base, indistinctly 2-segmented; without recognizable cerarii anterior to the 3 or 4 posterior abdominal pairs, the two posterior each with 2 spines, the antepenultimate with 2 spines or 1 spine and 1 spine-like seta or 2 spine-like setae, the next with 2 spine-like setae or not evident; none of cerarii underlaid by chitinized thickenings, triangular pores somewhat more numerous around each pair of spines, but hardly definitely clustered; apical cerarii each with as many as 11 setae above and around the spines, possibly to be considered as associated with it, but not intimately, remaining developed cerarii with as many as 4 or 5 setae adjacent to, but not intimately associated with, the spines; anal lobes not protruding, apical setae stout, about $175\ \mu$ long, somewhat longer than those of anal ring (these about $130\ \mu$), without any ventral chitinized thickening; anal ring normal, with inner and outer pore rows and 6 setae; ventral cicatrix small, transversely oval, the posterior side often bulging more or less distinctly; with the usual types of pores present, the triangular type more or less uniformly distributed over both surfaces, the multilocular disk type likewise present on both surfaces, very abundant in the posterior ventral abdominal region, less abundant but still numerous over the whole of the remainder of the ventral surface, much fewer and more widely scattered over the dorsal surface; ducts reduced to a few of the short, small tubular sort along the body margin; body setae fairly large and conspicuous for the genus, longest near body margins, and longer beneath than above, varying considerably in size.

This species has been redescribed from several specimens mounted from the Maskell material of "*calceolariae*," but without precise data, and from specimens from California on *Phormium*, with reference to Leonardi's description of *diminutus*. It appears from this material that there is definite variation in the number and development of the posterior cerarian spines, or perhaps more precisely, in the extent to which the tendency toward reduction and modification has affected these structures. The outstanding characteristic of the

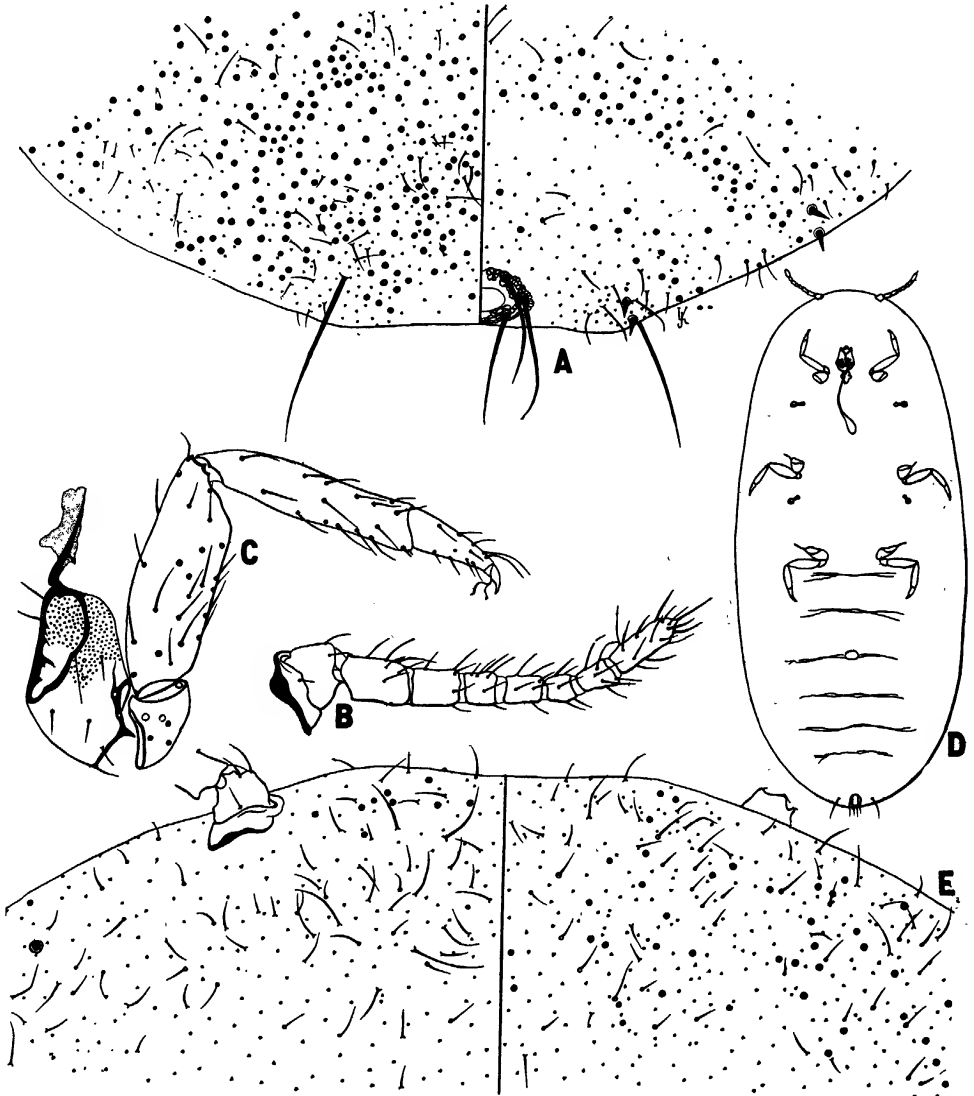


FIG. 6. — *Trionymus diminutus*, adult female: A, apex of abdomen, $\times 96$; B, antenna, $\times 96$; C, posterior leg, $\times 96$; D, outline of body, optical section, $\times 10$; E, apex of head, $\times 96$

pecies, in connection with the cerarian reduction, is the profuse development of the, normally, ventral abdominal, multilocular disk pores and their occurrence on the dorsum as well as over the whole ventral surface.

So far as positively known, the species is normally confined to *Phormium* as a host, although, as pointed out in discussing the intricacies of Maskell's utilization of the name *calceolariae*, the specimens recorded by him from *Cordyline australis* in 1893 may reasonably be given a tentative assignment here.

THE DIFFERENTIATION OF THE SPECIES INVOLVED

The key offered below, which will, it is hoped, facilitate the recognition of the species under discussion, is enlarged to include all of the species of mealybugs that have been recorded as occurring on sugar cane, in the belief that its usefulness will be greatly increased thereby. These additional species, so far as the available records show, are *Ferrisia virgata* (Cockerell), *Pseudococcus brevipes* (Cockerell) (*bromeliae* of most authors), *citri* (Risso), *saccharifolii* (Green), *Ripersia sacchari* (Green), and *Trionymus sacchari* (Cockerell). One record for *Pseudococcus maritimus* (Ehrhorn) is available, but here the species was taken in a greenhouse where the host was closely associated with a number of other plants capable of serving as hosts for it, so it is not considered a sufficiently normal record to justify the inclusion of the species in the key. Some other species of mealybugs have been reported from sugar cane in literature, but since the correctness of the identifications is not certain they are not included here. Usable descriptions and illustrations of the species *Ferrisia virgata* (Cockerell) (as *Pseudococcus*), *Pseudococcus brevipes* (Cockerell) (as *bromeliae*), and *Trionymus sacchari* (Cockerell) (as *Pseudococcus*) have been given by the writer (16, p. 171-175); *Ripersia sacchari* was described and figured in 1900 by Green (4, p. 37-38), as was *Pseudococcus saccharifolii* in 1908 (5, p. 23-24). Unless otherwise indicated, authentic specimens of each species included in the key which follows have been examined.

Key for separation of the species of mealybugs previously discussed

- a. Cerarian spines and indications of cerarii entirely wanting-----*Ripersia sacchari* Green ⁴
- aa. At least the apical abdominal pair of cerarian spines present, and that cerarius more or less distinctly developed.
 - b. Body with numerous large tubular ducts, each opening in a circular chitinized plate bearing setae, these ducts more or less clustered and in part replacing in position the anterior abdominal cerarii; only the apical pair of abdominal cerarii developed-----*Ferrisia virgata* (Cockerell)
 - bb. Without such large ducts opening through plates.
 - c. With only the apical cerarian spines present, these cerarii indistinctly developed; margins of abdominal segments, anterior to apical, each bearing a long seta approximating the apical seta in length; body very stout at maturity-----*Trionymus sacchari* (Cockerell)
 - cc. With two or more apical pairs of cerarii developed; without large marginal setae anterior to the apical pair; body more elongate.
 - d. No cerarian development on head; not more than four pairs of abdominal cerarii developed.
 - e. Only the two posterior pairs of cerarii developed; multilocular disk pores scattered very sparsely over ventral surface of body, numerous around genital opening only--*Trionymus danthoniae*, new species.
 - ee. With three or four posterior pairs of cerarii more or less developed; multilocular disk pores relatively very abundant over whole ventral surface and also sparingly present dorsally-----*Trionymus diminutus* (Leonardi)

⁴ Included from a study of the original description only; no specimens examined.

dd. At least one pair of cerarii present on head between antennae; at least four, and usually more, distinctly developed pairs of abdominal cerarii present.

f. Apical abdominal cerarii each normally with more than two spines.

g. Apical abdominal cerarii each normally with four spines; series of cerarii incomplete, one cephalic and five abdominal pairs present.

----- *Pseudococcus ambiguus*, new species

gg. Apical abdominal cerarii each normally with eight spines; series of cerarii complete, present on both thorax and abdomen.

----- *Pseudococcus saccharifolii* (Green).⁵

ff. Apical abdominal cerarii each normally with only two spines.

h. Series of cerarii incomplete, only one pair on the head and the five or six posterior abdominal pairs developed, all the abdominal with not more than two spines.

----- *Pseudococcus borinensis* (Kuwana)

hh. Series of cerarii complete, at least 17 pairs present.

i. With 18 pairs of cerarii, each with two spines; ventral chitinized thickening of anal lobes elongate, nearly linear, apical seta of anal lobes about twice length of anal ring setae.

----- *Pseudococcus citri* (Risso)

ii. With 17 pairs of cerarii, some of these with more than two spines; apical seta of anal lobes not much longer than anal ring seta.

j. Some, at least, of intermediate abdominal cerarii with more than two spines in each; ventral chitinized thickening present, irregularly quadrate.

----- *Pseudococcus brevipes* (Cockerell)

jj. All abdominal cerarii normally with two spines in each; ventral chitinized thickening not noticeably developed.

----- *Pseudococcus calceolariae* (Maskell)

SUMMARY

The specific name *calceolariae* has, through misidentifications, been used in literature to designate several different species of mealybugs. Properly, its use must be restricted to specimens identical with those collected by Maskell at Christchurch, New Zealand, on *Traversia*, in 1878. The name *Trionymus danthoniae*, new species, should be applied to specimens from *Danthonia* from New Zealand previously identified as *calceolariae*; the name *Trionymus diminutus* (Leonardi) should be applied to specimens from *Phormium tenax* from several

⁵ Included from a study of the original description only; no specimens examined.

localities throughout the world, previously identified as *calceolariae*; the name *Pseudococcus boninsis* (Kuwana) should be applied to specimens of an elongate gray mealybug already known to occur on sugar cane in many parts of the world and previously identified as *calceolariae*. Some references in literature to the species *Pseudococcus saccharifolii* (Green) and *Trionymus sacchari* (Cockerell), both of which, from the information available, are distinct and valid species, actually refer to *Pseudococcus boninsis* (Kuwana). The variety *minor* (Maskell) of the species *calceolariae* is identical with *Pseudococcus citri* (Risso).

The various species involved under the name *calceolariae* may be recognized from the detailed descriptions and from the key given in the body of the paper, while all of the mealybugs definitely known to occur normally on sugar cane may be separated by this same key.

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PLATE COUNTS OF SOIL MICROORGANISMS¹

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INTRODUCTION

Although plate counts of the microorganisms in soil samples have only restricted value, nevertheless, improvements in the method for making such determinations are desirable. Recently recommendations have been made on three points, (1) procedure in preparing the suspension of the soil organisms, (2) the use of special media for plating, and (3) frequent repetitions of the platings. These recommendations have been tested experimentally, and the results are presented in this paper.

METHODS FOR PREPARING SUSPENSIONS OF SOIL MICROORGANISMS

The intensity with which soil samples suspended in water should be shaken has been tested repeatedly, and it was the general belief that thorough shaking by hand for about five minutes gives a satisfactory suspension. The efficiency of this method, however, has been questioned by Whittles (14),³ who subjected soil suspended in water to a high frequency vibration with an electric vibrator. On plating out from these vibrated suspensions, he obtained counts widely surpassing those obtained with the hand-shaking method. In his first experiments with a soil which gave about 10 million organisms per gram by the usual plating procedure, he counted 250 millions per gram soil from the vibrated suspension. Later platings with a modified technique gave even counts of 100 and 270 colonies per plate from the 1-10¹¹ dilution equal to 10 and 27 billion⁴ organisms per gram soil.

The colonies in this case attained their maximum growth within 4 days, and there were no "slow growers." Whittles suggested that this was due to the freeing of the bacteria from the enveloping colloidal gel which contained their metabolic products. The dispersion of the gel by the vibration was declared to have removed the inhibiting influence and to have caused the rapid growth. An "alkaline shake medium" containing a protective colloid (gelatin) was found to be helpful in bringing about a good dispersion. For comparison, direct microscopic counts were made in the soil samples. The numbers recorded (6,300 millions per gram) are much higher than those usually found in soil tests and agree well with the lower vibrator counts.

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² The writers are especially indebted to F. Löhnis for many helpful suggestions and for critical reading of the manuscript, and to F. L. Goll for the drawing of the curves.

³ Reference is made by number (italic) to "Literature cited," pp. 516.

⁴ In England and Germany the term billion is used for a million millions and not for a thousand millions as in America. Billion in this paper is a million millions.

It was obvious from the start that the high numbers recorded could not be correct. The volume of 1 gm. of soil is ordinarily a little less than 1 c. c. This space would be completely filled by approximately 1,000,000,000,000 (1 billion) bacteria (6 p. 17). The 10 or more billions⁴ per gram counted by Whittles undoubtedly originated from outside contaminations (11). But there still remained the possibility that after eliminating this source of error the use of the vibrator might insure a more perfect distribution of the soil organisms than is obtainable by shaking the soil dilutions by hand.

THE VIBRATOR APPARATUS

Whittles' vibrator apparatus consists mainly of four parts: A vibrator cup, an electric vibrator, a reservoir for sterile diluent, and a sterile receiving flask. The writers constructed a similar apparatus in the following manner.

The vibrator cup was made from a large-mouthed glass jar 2¼ inches in diameter, having an aluminum screw cap. The bottom of the jar was cut off, and the center of the cap removed, leaving only enough of it to make a good seal when screwed on. A disk of pyralin was fitted into this cap and a soft rubber gasket placed on each side to prevent leaking. When the jar is inverted the disk of pyralin becomes the bottom of the vibrator cup and the vibrating membrane. For the outlet, a hole was drilled in the side of the cup at the 100 c. c. level and a right-angle tube with a rubber gasket inserted.

The vibrator was simply an ordinary electric door bell with the bell removed. A rubber stopper was placed under the hammer so that by raising or lowering it, adjusting the screw of the make-and-break and the height of the vibrator cup above the hammer, the correct vibration of high frequency could be produced, violently agitating the surface of the liquid in the cup.

The reservoir was a 2-liter Erlenmeyer flask having a siphon long enough to reach nearly to the bottom of the vibrator cup. The writers found it most convenient to sterilize the filled reservoir for 1 hour in the autoclave at 20 pounds pressure. In order to be able to do this, the lower end of the siphon had to be flexible, so that it could be turned upward on itself, closed with a sealed rubber tubing, and wrapped in cotton. When ready for use, a screw clamp on the flexible portion controlled the flow through the siphon. The receiving flask was a sterile 2-liter flask graduated at 1,900 c. c.

STERILIZATION OF THE VIBRATOR

The sterilization of the vibrator cup, with its pyralin membrane and rubber gaskets, could not be carried out in the autoclave or hot-air oven, nor could salts of the heavy metals be used. Alcohol seemed to fulfill the requirements and was tried in the first experiments. The vibrator cup was taken apart, soaked for 20 minutes in 50 per cent ethyl alcohol, rinsed with sterile water, and assembled under aseptic conditions. It was then placed in position over the vibrator, fastened with a clamp, filled with sterile water, and the vibrator started. As a check on the sterility of the cup, four 1 c. c.

⁴ See footnote on p. 1.

portions were withdrawn and plated. One gram of a clay loam field soil was put into the cup, and after a minute or two of vibration the siphon from the reservoir was started. The flow was adjusted so that the 1,900 c. c. would pass through in from 20 to 30 minutes. At the end, the suspension was mixed and further dilutions were made in sterile tap water in steps of $10\times$, by putting 5 c. c. in 45 c. c. Four plates from the 1:200,000 dilution were poured on soil extract agar.⁵ As a check on the effect of the vibrator, 10 gm. of the soil were added to 1,000 c. c. sterile tap water and shaken vigorously from 3 to 5 minutes. Dilutions were made in steps of $10\times$ by adding 5 c. c. to 45 c. c., reaching the final dilution of 1:100,000 from which four plates were poured. (Data in Table I.)

TABLE I.—Results of the first plating, together with series 2 which was made as a control to series 1

Series	Suspension by—	Dilution	Millions per gram soil	Control from cup, per c. c.
1	(Hand.....)	1:100,000	7.5	-----
	(Vibrator.....)	1:200,000	14.6	• 5,000
2	(Hand.....)	1:100,000	13.1	-----
	(Vibrator.....)	1:200,000	22.6	4,200
	(Vibrator.....)	1:200,000	15.4	6,500

• Estimated.

A glance at the last column of Table I is enough to condemn alcohol as the sterilizing agent of the vibrator cup. Although the effect of this contamination on the final dilution is small, it is a fruitful source of error.

The next method of sterilizing consisted in soaking the parts of the vibrator cup in a solution of equal parts of formaldehyde and water for 15 minutes. The parts were then rinsed with sterile water, assembled aseptically, put in place over the vibrator, and filled with sterile water. At this point the gallic acid test for formaldehyde was made with 2 c. c. water from the cup.⁶ If the test was positive, sterile water was run through until it became negative. One gram of soil was then added, and vibrated as in the preliminary experiment.

The first trials with formaldehyde showed that the apparatus could be rendered practically sterile. It was the writers' custom, however, to make four plates from the vibrator cup before every set of experiments, thus positively checking on the sterilization. In 13 different tests, representing a total of 52 plates, an average of only 2 colonies per plate was found, the highest number recorded being 7, while many plates were sterile.

Whittles (14) used bleaching powder for the sterilization of the vibrator, and the writers tested this substance, too. Following his directions, the cup was taken apart, "thoroughly washed in running water under the tap, rinsed with a solution of bleaching powder to which a drop of hydrochloric acid had been added; it was again washed thoroughly in running water, and then with a dilute solution

⁵ Field soil 500 gms., water 1,200 c. c., autoclave half hour at 15 pounds, or boil over a bare flame for 15 minutes. Filter, make up to 1,000 c. c., add 0.5 gms. K_2HPO_4 and 20 gms. agar. Adjust to P_H 7 to 7.2.

⁶ To 2 c. c. sample add a few drops of a saturated alcoholic (absolute) solution of gallic acid and stratify over concentrated sulphuric acid. A blue ring indicates formaldehyde. Sensitive to 1 part in 200,000.

of sodium thiosulphate and again with distilled water; finally with some sterile shake medium.”⁷ Two soils were used for these tests. One gram in each case was vibrated with 2,000 c. c. diluent, then 2 c. c. of this suspension again vibrated with 2,000 c. c., making a final dilution of 1:2,000,000. The hand-shaking control plates of these soils gave 12 and 10 millions per gram soil, respectively. The analogous data for the vibrated suspensions were as follows: First test, 554 millions and 26 millions; second test, 47.5 millions and 41.2 millions.

Although these counts are much lower than those made by Whittles, they suffice to show that undoubtedly the error lay, in his case, in the incomplete sterilization of the vibration cup.

EFFICIENCY OF VIBRATION

After having ascertained that the use of formaldehyde assures a satisfactory sterilization of the vibrator, the writers’ attention was turned to the effect of the vibration and to the value of the “alkaline shake medium” recommended for making soil suspensions. The shake medium was prepared by diluting 50 c. c. of the stock solution (Na Cl 10 per cent : Na₂CO₃ 2.5 per cent) to 4 liters, which were sterilized. As inoculum two clay soils were chosen (Nos. 109 and 260). These had been in the laboratory for more than a week. Methods of making dilutions and plating were the same as in the preliminary experiments. The results of the nine tests are presented in Table II.

TABLE II.—Influence of vibration and “shake media” on total counts

Suspension by—	Dilution	Sterile diluent	Soil 109 millions per gram	Soil 260 millions per gram
Hand shaking.....	1 : 250, 000	Tap water.....	16. 7	13. 0
Vibrator.....	1 : 200, 000do.....	18. 8	12. 6
Do.....	1 : 200, 000	Whittles' shake medium.....	16. 6	11. 8
Do.....	^a 1 : 2, 000, 000do.....	^a 13. 0	^a 20. 0
Do.....	^a 1 : 2, 000, 000	Whittles' medium, plus 0.1 per cent gelatin.....	^a 18. 0	^a 18. 0
Do.....	1 : 200, 000	Whittles' shake medium, plus 0.1 per cent agar.....	15. 9	11. 3
Do.....	1 : 200, 000	Whittles' shake medium, plus 0.1 per cent gelatin.....	11. 1	10. 6
Hand shaking.....	1 : 250, 000	Tap water.....	11. 6	10. 1

^a Dilutions were made by placing 2 c. c. of the first dilutions back into the vibrator cup and repeating the vibration. The colonies were not numerous enough for accurate counting.

These analyses extended over a period of several days and were made in the order given in the table. That there was very little change in the total number of microorganisms is shown by the close agreement of the hand-shaking control made at the beginning and at the end of the series. If the counts from the 1:2,000,000 dilutions, which were too high, are excluded, the same agreement is evident throughout the table, whether a “shake medium” or tap water was used as diluent, and whether the suspension was made by the hand-shaking or by the vibrator method. Furthermore, the addition of a protective colloid to the suspension, such as 0.1 per cent gelatin or agar, gave no higher counts than when sterile tap water was used as diluent.

⁷ Nothing is said in these directions about the necessity of using not simply “distilled water,” but “sterilized distilled water.”

All the counts given in Table II were made on plates poured with soil extract agar. As Whittles used a "mannite-salts agar" (14, p. 19), it was necessary to adopt this medium to get fully comparable results. At the same time, it seemed desirable to check up the results given in Table II as to the efficiency of the vibration compared with hand shaking and also in regard to the influence of the diluent. The inoculum in this case was a clay-loam field soil.

TABLE III.—Comparison of mannite-salts and soil-extract agars, tap water and shake medium, vibration and hand shaking

Suspension by—	Dilution	Diluent	Mannite salts agar	Soil extract agar	Average	Average
			Millions per gm.	Millions per gm.		
Hand shaking.....	1 : 250,000	Tap water.....	8.9	10.8	9.9	9.7
Do.....	1 : 250,000	Shake medium.....	8.3	10.6	9.5	
Vibrator.....	1 : 200,000	Tap water.....	11.5	16.6	14.0	11.9
Do.....	1 : 200,000	Shake medium.....	7.7	11.8	9.8	
Average.....			9.0	12.4		

A comparison of the counts made on mannite-salts agar and on soil-extract agar, as recorded in Table III, shows a small but distinct difference in every case in favor of soil-extract agar. With the soil-extract agar, the colonies showed less tendency to spread, and on the whole they were easier to count. Tap water used as diluent gave consistently higher counts, although the difference is small in the hand-shaking series. When vibrator and hand shaking are compared, the former gave a somewhat higher average, but the counts making up this average are much more erratic than those in the hand-shaking series, and consequently less valuable.

In this connection, the fact should not be overlooked that growth might take place in the vibrator or in the suspension. When making the experiments, not less than 45 minutes elapsed between the addition of the soil to the vibrator cup and the plating out of the highest dilution. In order to ascertain whether growth actually amounts to much under these conditions, the following test was made. A three-day-old agar slant culture of *Bacterium fluorescens* was flooded with sterile water, the growth scraped off, and this suspension transferred to a flask of sterile water. After shaking for three minutes, four aliquots were taken out, two of which were diluted by hand shaking, one in tap water and the other in shake medium with gelatin; and two were vibrated with the same diluents. Plates were poured from the 2 and 2,000,000 dilutions, incubated at 28° C. and counted after three days with the following results:

1. Control, diluted with tap water, 600 millions.
2. Control, diluted with shake medium (gelatin), 540 millions.
3. Vibrated, diluted with tap water, 135 millions.
4. Vibrated, diluted with shake medium (gelatin), 690 millions.

The vibrator cup leaked at the beginning of the first run and some of the inoculum was lost; therefore, the count of 135 millions can be disregarded. In spite of this accident, it can safely be assumed that growth is not responsible for a marked increase, especially if only one suspension is made in the vibrator, as in the case just given.

In making the suspensions, the even dispersion of the soil particles by the vibrator was very evident. The suspensions did not contain any of the large particles that were common in the hand-shaking series, and on the whole the suspensions seemed more uniform. This might be expected, since there was no current to carry over the heavier particles and only those particles which were dispersed by the vibration could pass into the receiving flask.

The residue in the vibrator cup naturally varied with the sample. Of 1 gm. of soil No. 109, 0.58 gm. remained in the cup, 0.42 gm. having become suspended in the diluent. For soil No. 260, the figures were 0.38 gm. and 0.62 gm., respectively.

MODIFIED MEDIA FOR PLATING

Preliminary tests made some years ago with Conn's asparaginate agar (1), variously modified media as recommended by Lipman and Brown (4), and soil-extract agar (5, *p.* 101), indicated that the highest and most uniform counts were obtained on soil-extract agar. This latter medium was therefore adopted in this laboratory for making routine counts of the microorganisms in the soil. The mannite-salts agar used by Whittles was originally recommended by Thornton (10). Three advantages were ascribed to its use—that it inhibits spreading colonies, that it is uniform in composition, and that the results can be produced with different batches of the medium. Unfortunately, no comparative results obtained with a known medium have been presented, so that its efficiency, aside from its ability to repress spreading colonies, remained in doubt. The few tests recorded in Table III were not very promising, but it seemed desirable to use this medium along with others in making routine platings of a series of soil samples taken from small squares at frequent intervals.

COMPARISON OF MANNITE-SALTS, EGG-ALBUMEN, AND SOIL-EXTRACT AGARS

Dilutions for plating were made by adding 10 gms. of soil to 250 c. c. sterile tap water, and, after vigorous shaking for about three minutes, 10 c. c. of the suspension were added to 90 c. c. of sterile water. This was repeated until a dilution of 1:250,000 was reached. One cubic centimeter of this dilution was then pipetted into the Petri dish, melted agar was poured in, and the plates were incubated for seven days at 28° C.

The second part of Table IV contains the results of the platings made August 13 to 27 from squares 21 to 46, inclusive, on soil-extract (S. E.) and mannite-salts agars (M. S.).⁸ Four plates were poured with each medium, the figure for each square being the average of these four plates in millions of microorganisms per gram of dry soil. The counts on soil extract agar (S. E.) are from 2 to 6 times higher than those on the mannite-salts agar (M. S.), the average of the 26 tests being 30.1 and 8.0, respectively.

⁸ The mannite-salts agar is made as follows: K_2HPO_4 , 1 gm., KNO_3 , 0.5 gm., asparagine, 0.5 gm. Dissolve in water, and add 0.2 gm. $MgSO_4$, 0.1 gm. $CaCl_2$, 0.1 gm. $NaCl$, and 0.002 gm. $FeCl_3$ in the form of standard solutions. Fifteen grams agar is then added and dissolved, the volume is made up to 1,000 c. c., and the agar is filtered at 100° C. through cotton. One gram of mannitol is added to the filtrate, and this is cooled to 60° C., and adjusted to PH 7.4. Sterilization in the autoclave.

TABLE IV.—Results of frequent platings

Date	Mean temperature	W. H. C.	Square No.	Millions per gram		Date	Mean temperature	W. H. C.	Square No.	Millions per gram		
				H.	V.					S. E.	M. S.	E. A.
	° F.	Per cent					° F.	Per cent				
July 26.....	72	25	1	29.2	27.2	Aug. 28....	75	33	47	26.7	3.0	20.1
27.....	72	25	2	27.5	17.4				48	28.7	5.1	16.8
30.....	76	45	3	29.5	27.9	29.....	74	40	49	29.2	3.9	16.7
31.....	69	53	4	31.2	19.1				50	19.7	4.3	18.5
Aug. 1.....	67	57	5	32.5	27.9	30.....	74	41	51	28.6	5.4	21.9
2.....	75	60	6	38.5	25.0				52	26.1	5.2	20.4
3.....	82	47	7	39.3	34.4	31.....	74	45	53	24.4	5.4	17.0
4.....	82	50	8	30.5	32.9				54	29.2	5.7	22.0
6.....	81	43	9	28.3	36.2	Sept. 1....	73	40	55	16.4	2.3	17.0
7.....	84	43	10	28.1	30.3				56	22.0	3.3	20.1
Average.....			11	32.9	29.1	4.....	78	40	57	19.7	2.3	16.7
			12	25.1	25.1				58	20.0	1.8	19.0
			13	33.3	30.5	5.....	73	41	59	25.7	5.3	19.5
			14	36.5	33.5				60	24.6	5.0	17.1
			15	30.4	32.6	6.....	68	60	1	27.8	3.1	29.4
			16	28.1	36.0				2	24.4	2.1	27.2
			17	30.5	29.6	7.....	74	60	3	27.5	2.2	25.2
			18		30.6				4	32.3	2.0	27.5
			19	26.6	23.6	8.....	72	55	5	24.3	3.2	20.8
			20	35.3	21.0				6	29.7	4.4	19.0
				31.2	28.5	10.....	65	50	7	24.0	3.7	21.7
				S. E.	M. S.				8	26.7	3.4	18.1
Aug. 13.....	74	43	21	29.0	12.6	11.....	65	50	9	25.1	4.5	18.4
14.....	74	37	22	31.8	15.5				10	21.8	4.4	17.8
15.....	80	37	23	26.7	11.7	12.....	71	48	11	26.1	5.4	20.9
16.....	73	37	24	23.8	9.0				12	25.0	6.0	18.2
17.....	65	37	25	25.2	5.6	13.....	70	43	13	23.8	4.7	27.5
18.....	67	63	26	26.5	6.0				14	33.7	5.4	23.6
20.....	74	47	27	24.4	9.8	14.....	59	43	15	25.1	8.5	26.8
21.....	76	47	28	34.0	11.4				16	27.4	9.2	23.6
22.....	65	43	29	25.8	7.0	15.....	57	40	17	27.8	4.4	25.1
23.....	62	40	30	21.3	6.5				18	21.8	5.8	17.5
24.....	66	45	31	31.5	7.5	17.....	58	37	19	30.4	8.1	25.0
25.....	70	47	32	38.5	7.4				20	20.9	6.6	16.5
27.....	76	43	33	30.4	5.7	18.....	60	32	21	16.6	3.5	20.2
Average.....			34	31.5	8.0				22	21.7	3.9	17.8
			35	30.0	5.8	19.....	74	33	23	24.4	5.8	18.1
			36	30.5	6.6				24	21.6	7.7	18.1
			37	34.6	15.0	Average.....				25.0	4.6	20.7
			38	31.7	8.2					28.0		
			39	33.9	7.0	Average.....	72	44				
			40	35.7	8.5							
			41	30.4	6.4							
			42	32.0	4.4							
			43	29.9	5.1							
			44	30.3	5.1							
			45	33.4	5.2							
			46	30.2	5.2							
Average.....				30.1	8.0							

• H. = horizontal sampling. V. = vertical sampling. S. E. = soil extract agar. M. S. = mannite salts agar. E. A. = egg albumen agar. W. H. C. = water-holding capacity.

In plating another series of samples (Nos. 47 to 60 and 1 to 24), taken from adjoining squares on August 28 to September 19, egg-albumen agar (E. A.) was also used. The formula of this substrate as given by Waksman (13) is as follows: K_2HPO_4 0.5 gm., $MgSO_4$ 0.2 gm., dextrose 10 gms., $Fe_2(SO_4)_3$ trace, and agar 15 gms., dissolved in 1,000 c. c. distilled water; then 0.25 gm. egg albumen added, dissolved in 0.1 NaOH. The counts obtained on the three media are recorded in the third part of Table IV. During the progress of these tests several batches of the three media were made up and found to be uniform. The PH of each was within 0.2 of neutrality after sterilization.

Soil-extract agar again shows its superiority, while the mannite-salts agar, although a fairly good substrate for some of the soil organisms, inhibits the development of a large group of bacteria (2). The counts on egg albumen were consistently lower than on soil-extract agar, as Waksman (13) also observed. His results show that soil-extract agar gave the highest count, casein agar came next, and this was followed by egg-albumen agar. If the counts obtained by him on soil-extract agar are recorded as 100, those on casein and egg-albumen agar equal 93 and 73 per cent, respectively. Despite this decided advantage in favor of soil extract, it was recommended that egg-albumen agar be adopted for the determination of the total counts of the microorganisms in soil, and that soil-extract agar should be discarded because of its being "not standard in composition." Others have raised the objection that soil extract is of unknown composition, and that its variability would make it impossible to compare results obtained by workers experimenting with different soils. To test the validity of this objection, the following experiments were made.

COMPARISON OF DIFFERENT SOIL-EXTRACT AGARS

Ten soils, ranging from a very rich muck to a loamy sand, were selected for preparing the extracts. It was realized at the outset that these soils presented greater differences in character than would be found in field soils likely to be used for making extracts. But since so much weight has been attached to the assumed variability of soil extracts, these writers attempted to cover wide range in one experiment. The soils used were the following, with name and source of sample, and with the soil-survey party's characterization when the sample was taken.

Muck.....	Ontario, N. Y.....	Very rich.
Peat loam.....	Walliston, Va.....	Do.
Greenhouse soil.....	Washington, D. C.....	Clay loam, sand, and compost.
Rich lawn.....	do.....	Clay loam.
Granby loam.....	Sodus, N. Y.....	Dark, heavy, poorly drained.
Genesee silty clay loam.....	Lyons, N. Y.....	Rich bottom land.
Silty clay loam.....	Arlington, Va.....	Field soil, low in nitrogen.
Hollis loam.....	Ludlow, Vt.....	Light loam, fairly fertile.
Dunkirk fine sandy loam.....	Ontario, N. Y.....	Light, low in humus.
Coloma loamy sand.....	Taunton, Mass.....	Very poor.

The samples were all taken from cultivated fields, and air-dried. Extracts were made in a uniform manner, 500 gms. soil being boiled about 15 minutes over an open flame with 1,200 c. c. water, filtered through paper, and the volume made up to 1,000 c. c. Agar 2 per cent and K₂HPO₄, 0.05 per cent, were added to the extracts, and the reaction was adjusted to approximately PH 7.0.

Table V shows the results of plating 5 representative soil samples on soil-extract agars made from these 10 soil types as well as on Thornton's mannite-salts agar and on egg-albumen agar. Samples 1 and 2 were taken from a lawn (squares 25 and 26, Table IV), sample 3 came from a field at Arlington Experiment Farm, Rosslyn, Va., samples 4 and 5 from two rows of pots (Nos. 34 and 35) in a greenhouse experiment. A dilution of 1:250,000 was used in plating samples 1, 2, and 3, but 1:750,000 with 4 and 5.

TABLE V.—*Influence of various soil-extract agars on the number of microorganisms growing on the plates*

Soil-extract agar made from—	Millions per gram soil					
	1	2	3	4	5	Average
Muck.....	30.5	43.5	18.6	128	197	83.5
Peat loam.....	31.2	37.8	14.7	137	252	94.5
Greenhouse soil.....	30.4	33.9	14.6	152	252	96.6
Rich lawn soil.....	29.0	39.4	15.5	135	238	91.3
Granby loam.....	31.6	34.3	17.4	116	216	83.0
Genesee silty clay loam.....	33.7	42.8	19.1	140	263	99.7
Silty clay loam.....	36.8	44.3	18.0	149	260	101.6
Hollis loam.....	24.9	33.5	13.7	94	207	74.5
Dunkirk fine sandy loam.....	22.6	31.1	13.5	143	220	86.0
Coloma loamy sand.....	25.2	32.1	13.1	122	233	85.0
Egg-albumen agar.....	26.7	34.1	16.2	66	181	65.0
Mannite-salts agar.....	6.7	16.6	7.4	-----	65	23.9

^a Spreaders.

The data in Table V are very instructive. The fluctuation in the counts throughout the central part of the table is not greater than would be the case if several samples were plated on the same medium, but the appearance of the colonies on the different soil-extract agars varied considerably. Those agars containing larger amounts of organic matter were more favorable for the development of rapidly spreading colonies, especially of fungi. On the other hand, those made from light soils and containing a relatively small amount of soluble material, did not allow the development of the same number of colonies. Egg-albumen agar gave counts comparable to those on soil-extract agar when used for plating field soils, but in the case of the rich greenhouse soils its efficiency was only 50 per cent and 75 per cent. Thornton's mannite-salts agar again proved to be a rather poor substrate.

OTHER MEDIA FOR PLATING

Conn (1) made total counts of bacteria in various type soils by using as plating media Lipman and Brown's "synthetic" agar, Brown's albumen agar and Fischer's soil-extract agar, in comparison with soil-extract gelatin and asparaginate agar. From his data he concluded that soil-extract gelatin and asparaginate agar^a were the best for plating, but on account of the indefinite composition and the difficulty of handling gelatin, he declared asparaginate agar preferable. Accordingly, in plating out some samples of a field soil, the writers used soil-extract gelatin and asparaginate agar, together with a mannite-nitrate soil extract, and a "synthetic" agar. The mannite-nitrate agar was made by adding 1 per cent mannite and 0.02 per cent KNO₃ to soil-extract agar. The synthetic agar consisted of 2 per cent agar dissolved in distilled water containing 0.05 per cent K₂HPO₄, 0.1 per cent sodium asparaginate, 0.25 per cent sodium citrate, and 0.1 per cent sodium albuminate.

Table VI shows the value in these experiments, of these media for making total counts of microorganisms in the soil. Again, soil-extract agar proves superior. Although these tests are not numerous

^a The composition of asparaginate agar was: 1,000 c.c. distilled water; 12 gms. agar; 1 gm. sodium asparaginate; 1 gm. dextrose; 0.2 gm. MgSO₄; 1.5 gm. NH₄H₂PO₄; 0.1 gm. CaCl₂; 0.1 gm. KCl; trace FeCl₃.

enough for drawing any definite conclusions, they are of interest when considered along with the others just given.

TABLE VI.—Plate counts on different media, millions per gram of soil

Soil sample No.	Aspar- aginate agar	Soil- extract gelatin	Synthet- ic agar	Mannite- nitrate agar	Soil- extract agar
1.....	7.3	7.0	11.0	10.0	18.7
2.....	3.5	3.3	10.3	7.6	10.7
3.....	4.0		8.1	8.0	14.4
4.....	1.5		5.0	3.0	9.0
Average.....	4.1	5.1	8.6	7.1	13.2

FLUCTUATIONS IN PLATE COUNTS OF SOIL ORGANISMS

The fact that there is a seasonal rise and fall in the number of soil organisms has long been known. Daily or hourly fluctuations, however, have been studied only recently. Cutler, Crump, and Sandon (3) made 365 daily consecutive determinations of the bacteria and protozoa in a field soil which for a long time had received 14 tons of barnyard manure per acre annually. They observed great daily fluctuations in the numbers of bacteria, and noticed in many cases an inverse ratio between bacteria and protozoa. Soil moisture and temperature seemed to have no correlation with these fluctuations. Fortnightly averages showed the usual seasonal rise and fall and a marked parallelism in the numbers of bacteria and protozoa.

More recently the work of Cutler et al. has been continued at Rothamsted, and similar fluctuations have been observed at intervals of two hours. (8)

From the results obtained, it has been concluded that the usual method of making plate counts at intervals of one or several weeks does not give accurate information. Since, however, such wide fluctuations as recorded by the British investigators, as a rule, have not been recorded in bacteriological soil tests, and since, on the other hand, the fortnightly averages of those irregular counts agree closely with determinations made at longer intervals, it seemed advisable to make a series of comparative tests at frequent intervals for several weeks.

THE EXPERIMENT PLOT AND METHODS OF SAMPLING

The soil selected for sampling was a level piece of fertile lawn, 10 feet square. The sod was removed and the earth spaded to a depth of about 6 inches. Small stakes were placed a foot apart around the edge, and strings were drawn across the plot in both directions, thus laying out 100 squares (fig. 1). In numbering them, the outside row around the entire plot, as well as the inside corner squares, were left as a border, from which no samples were taken.

The test was started two weeks after spading. The vertical and the horizontal methods were used in taking samples. The former is the usual method of taking several borings for a mixed sample. In the horizontal method, about 3 inches of the surface soil were scraped off with a clean spade or trowel, and the sample taken from the underlying layer about 2 inches in thickness and extending over an area of about 10 square inches. By careful manipulation, both

methods could be carried out on the same square. One even-numbered and one odd-numbered square were sampled each day, except Sundays and holidays, plates were poured with soil-extract agar and other media for comparison, and the colonies were counted after seven days at 28° C.

In the first part of Table IV (July 26 to August 7, squares 1 to 20), the counts of the microorganisms are given as found in samples taken by the two methods. The average for horizontal sampling is 31.2 millions, that for the vertical method 28.5 millions.

If the odd and even squares representing the opposite sides of the experimental plot are averaged separately, the same numbers are obtained with the horizontal method of sampling (31.3 and 31.2 millions per gram). But in the case of vertical sampling, the odd squares have an average of 29.8 and the even squares 27.1 millions per gram.

Furthermore, the deviation of each count from the average is greater with the vertical method, the latitude being -11.1 to +7.7 millions for the vertical, and -4.6 to +8.1 millions for the horizontal.

These results indicate that greater uniformity as well as slightly higher counts are obtained when samples are taken by the horizontal method. It is well known that the greatest activity of the microorganisms occurs a few inches below the surface, and it seems preferable to take the samples

from this plane without the admixture of the less densely populated soil from higher and deeper layers. In sampling with an auger it is very difficult to get equal amounts of soil from the various layers. Thoroughly mixing a soil sample is undoubtedly of some value, but it does not mix the bacteria sufficiently to assure uniform distribution of them. In view of these facts, the horizontal method alone was used throughout the remainder of the tests.

RESULTS OF FREQUENT PLATING

From July 26 to September 19, 84 squares (i. e., squares 1 to 60 and 1 to 24 inclusive) were sampled and plated on soil-extract agar and the two other substrates just discussed. The counts for each square are given in Table IV. The average for the entire series was 28 millions per gram of soil (counted on soil-extract agar). If this is taken as 100, the counts for the odd and even squares sampled on the same day exhibit certain plus or minus percentage fluctuations from the general average, as shown by the third graph of Figure 2.

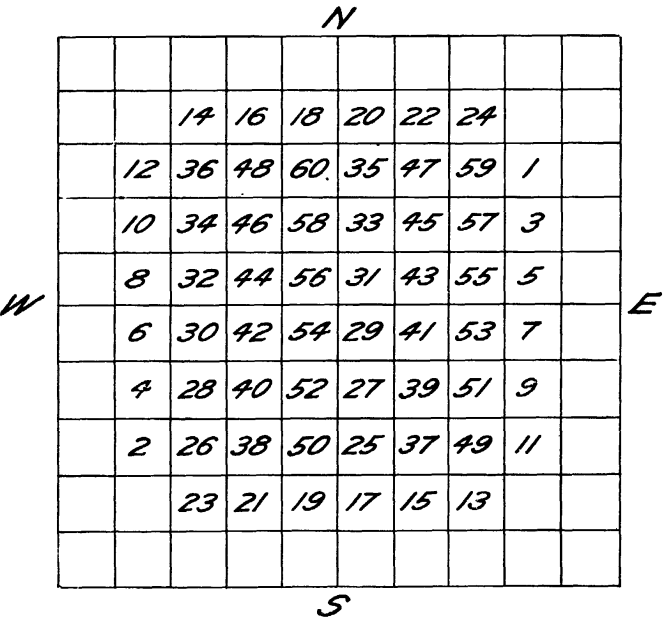


FIG. 1.—Experimental plot showing the squares in the order of sampling

The range of the fluctuations during the first half of the tests was between 25 per cent above and 15 per cent below the general average. During the second half there was a falling off in the numbers, with a fluctuation between 8 per cent above and 30 per cent below. In only two cases was the fluctuation from one determination to the next more than 25 per cent, even though one or more days had passed.

The fourth graph in Figure 2, showing the sample deviation on soil-extract agar, was made by taking the plate counts of the odd-numbered squares on each day as 100, and computing the percentage deviation of the counts of the even squares. Comparison of this graph with the preceding one shows that the variation between odd

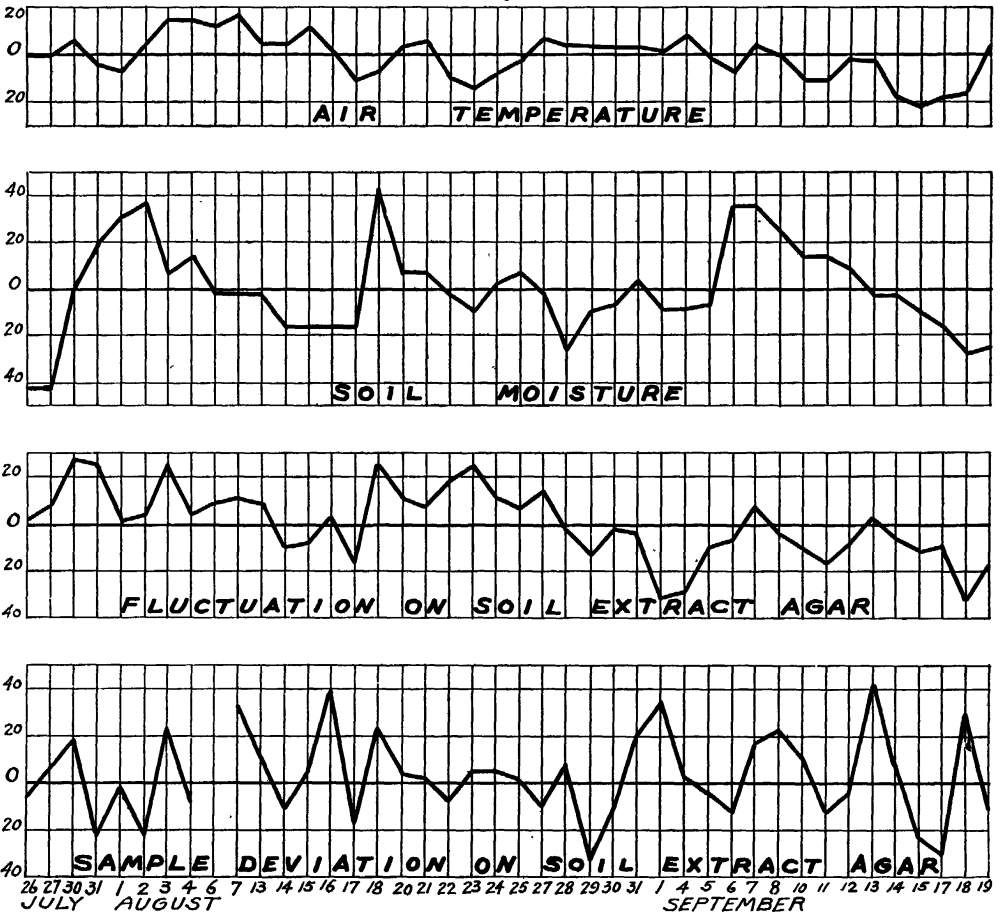


FIG. 2.—Graphic representation of plus and minus percentage fluctuations from general average of plate counts, counted on soil-extract agar

and even samples is greater than the fluctuations in the counts from one day to the next. But the averages of the odd and even squares for the entire series are practically identical, 27.9 and 28.2, respectively, showing that the experimental plot as a whole was very uniform and that the irregularities in the counts were most probably due to variation in the individual samples or unavoidable analytical errors.

The uniformity of the soil throughout the experimental plot can be further demonstrated by combining and averaging the counts from every second square across the plot, irrespective of the date of sampling (Table VII).

TABLE VII.—Average counts of combination of squares demonstrating the uniformity of the soil

Squares	Average	Squares	Average
1-47-60-36.....	27.8	59-35-48-12.....	27.4
3-45-58-34.....	28.6	57-33-46-10.....	27.1
5-43-56-32.....	30.7	55-31-44-8.....	27.2
7-41-54-30.....	30.0	53-29-42-6.....	30.2
9-39-52-28.....	30.6	51-27-40-4.....	30.0
11-37-50-26.....	28.4	49-25-38-2.....	28.4
Average.....	29.3	Average.....	28.4

The curves for air temperature and soil moisture are included in Figure 2 for comparison with the plate counts. The averages again are calculated as 100 and the daily fluctuations computed accordingly. The moisture curve shows three large fluctuations which can be likewise recognized in the plate-count curve whose gradual falling off is in general agreement with the analogous tendency of the temperature curve. In addition, slight soil differences have undoubtedly contributed to the special deviations of the plate-count curves.

As counterpart to the results obtained on soil-extract agar, curves representing the plate counts of the same samples on mannite-salts agar are shown in Figure 3. The fluctuations as well as the sample deviations are much greater on this medium, the former varying from 133 per cent above to 66 per cent below the average. Two factors may have been mainly responsible for this result—the selective character of the medium, and the smaller counts obtained, as shown in Table IV.

For comparison with these writers' results, a graph of results obtained at the Rothamsted Experiment Station has been included in Figure 3 showing the percentage fluctuations in the counts made by Cutler and his collaborators (3) on corresponding days of the year and using the same mannite-salts agar. It is interesting to note that the majority of the counts fall within the range of +20 per cent to -40 per cent and compare well with these present findings with the same medium, but they, too, are not as uniform as when soil-extract agar was used.

After the completion of these present experiments of the writers, Matthews (7), working at Rothamsted on the partial sterilization of soil by antiseptics, has reported that "the large number of counts made as controls on untreated soils failed to show, under the conditions of the experiment, any great saw-edged curve such as Mr. Cutler has proved for soils under field conditions." The counts were made in this case on gelatin plates.

PLATE COUNTS FROM COMPOSITE SAMPLES

In making plate counts of soil organisms it is customary to make one series of dilutions, usually starting with 10 gms. of soil in 100 or several hundred cubic centimeters of water. Several plates are then poured from the highest dilution, three being probably the most common number; Thiele (9) recommended 20, and Waksman (12) used 10 plates. Other workers have used from 2 to 10 plates to get a fair average, and have usually started from one composite sample in one series of dilutions.

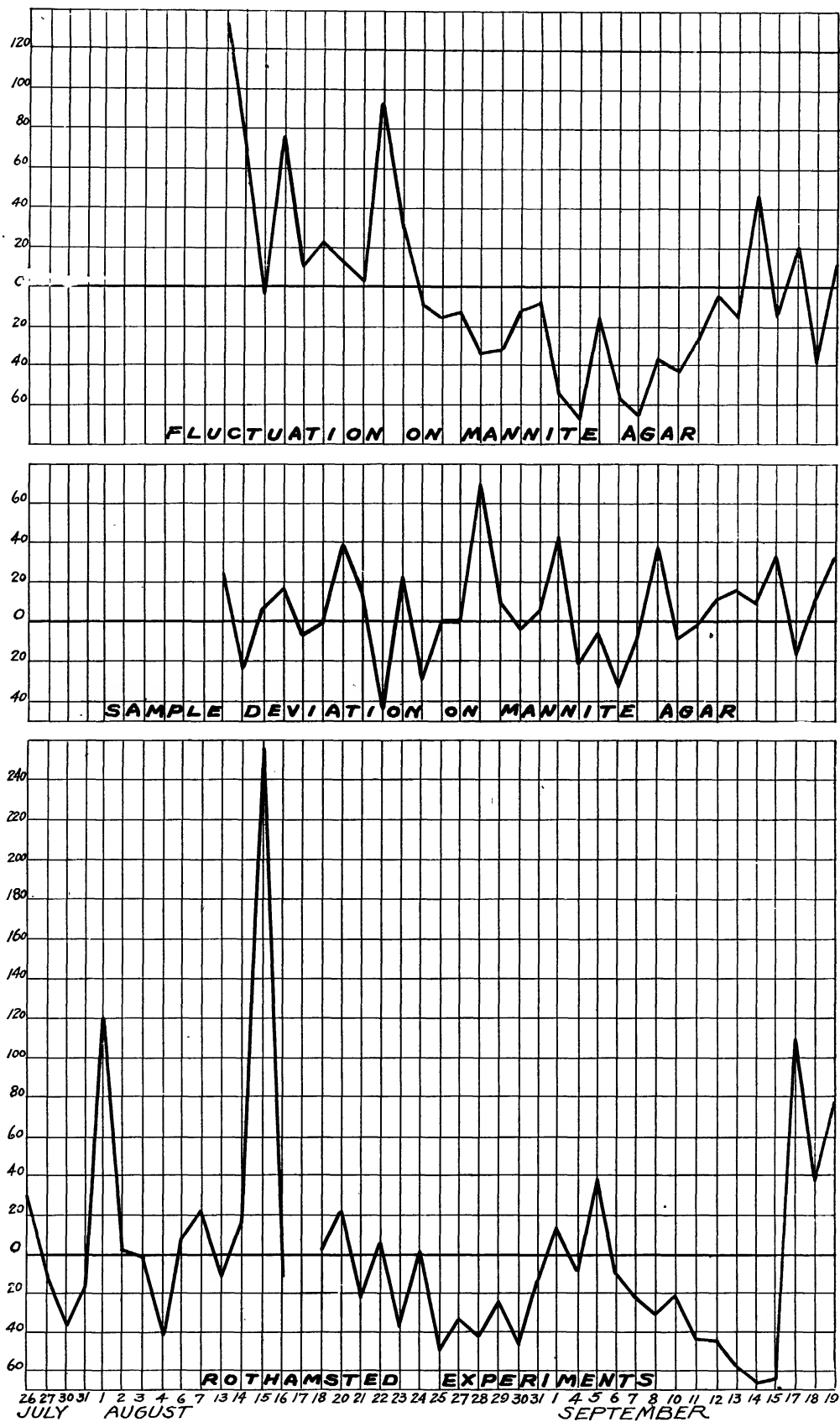


FIG. 3.—Graphic representation of plate counts of soil samples on mannite-salts agar

It remained to be seen whether duplicate series of dilutions would give concordant results. Four composite samples were taken from the plot used for the frequent platings, and two series of dilutions were made from each. From the highest dilution of each series, four plates were poured with soil extract agar and incubated for the usual seven days at 28° C. In Table VIII are given the averages of the four plates made from duplicate dilutions of four samples. The irregularity of the counts indicates that it is necessary to make parallel dilutions if a fair average is to be obtained. The same end may be attained, of course, by plating two or more samples in the usual way and taking the averages as single determinations. This is especially to be recommended if the field has been heavily fertilized and the soil is therefore of uneven structure.

TABLE VIII.—Plate counts from duplicate dilutions of composite samples

Sample	Dilution		Per cent deviation a from b
	a	b	
1.....	54.0	60.5	-11
2.....	104.0	80.5	+22
3.....	73.5	78.0	-6
4.....	120.0	91.0	+24

From a plot ($\frac{1}{20}$ acre) which had received each year 320 pounds sodium nitrate per acre in addition to minerals, Waksman (12) took 51 single samples at the same time. The counts varied from 8.6 to 22.6 millions per gram (average 12.9 millions), which result illustrated the well-known fact that one plating of a single sample is very unreliable. On the other hand, 5 composite samples consisting of 3 borings, each taken from a check plot which had not been fertilized for 14 years, gave very consistent counts, ranging from 7.39 to 9.07 millions (average 8.35). The close agreement of these counts, and the wide variation of those made on the single samples, should not be attributed to the method of sampling alone, as the different treatment of the plots has evidently influenced the results obtained.

That plate counts made from a very uniform soil may show fluctuations from the average of about 20 per cent plus or minus, even though composite samples are used, may be seen from the following test. In a greenhouse experiment, corn was raised in buckets filled with 25 pounds of an evenly mixed unfertilized soil. The average dry weights recorded for eight rows, each containing eight buckets, together with the plus and minus percentage deviations from the average, as given in Table IX, clearly show the uniformity of the soil.

TABLE IX.—Fluctuations in plate counts of uniform greenhouse soil

Row No.....	5	9	11	17	23	27	29	35
Dry weight, gm.....	13.0	13.3	13.4	13.5	13.1	12.8	13.1	13.0
Per cent \pm average.....	-1.5	+0.8	+1.5	+2.3	-0.8	-3.0	-0.8	-1.5
Total counts, millions.....	75.0	68.0	62.0	75.5	53.0	68.5	59.5	64.0
Per cent \pm average.....	+14.0	+4.0	-7.0	+14.5	-19.0	+4.0	-9.0	-4.0

From these rows, composite soil samples were taken, and plates were poured on soil-extract agar from one series of dilutions. The counts showed the usual fluctuations from plus 14 per cent to minus 19 per cent, but no relation to the percentage variation of the dry matter of the crop. If each component of the composite samples had been plated and the average calculated on such a basis, undoubtedly the variation of the counts would have been much less, but the work involved made such a procedure impracticable.

SUMMARY

The suspension of the bacteria in a soil sample may be effected by means of an electric vibrator of high frequency.

Plate counts made from such a suspension and from a suspension made by hand shaking gave concordant results.

Higher numbers obtained by the vibrator method would seem to indicate either incomplete sterilization of the vibrator or incomplete dispersion by hand shaking.

The greater difficulty of manipulating the vibrator precludes its use as a routine procedure.

Plates poured with soil-extract agar gave higher and more uniform results than those poured with other media. Egg-albumen agar gave lower counts, but was in general comparable to soil-extract agar. Mannite-salts agar proved to be too selective, and gave, accordingly, too low counts. Spreading colonies were equally rare on all these media.

Asparaginate agar, synthetic agar, soil-extract gelatin, and other media were all inferior to soil-extract agar.

Plate counts on soil-extract agars made from type soils ranging from loamy sand to muck showed that field soils of fair fertility, irrespective of their general character and location, are suitable for making soil-extract agars to be used in estimating the total number of soil microorganisms.

Plate counts of soil samples taken horizontally from a level approximately 4 inches below the surface are slightly higher and more uniform than those of samples taken vertically with an auger.

Fluctuations of 20 per cent above or below the average total count may occur with a uniform soil, if one series of dilutions is made and soil-extract agar used as plating medium. A more selective medium, such as mannite-salts agar, gave much wider fluctuations.

Duplicate series of dilutions of a composite soil sample showed similar fluctuations. Therefore, to be of any value, a total count should represent the average of three or more separate counts.

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CLADOSPORIUM LEAF MOLD OF TOMATO: FRUIT INVASION AND SEED TRANSMISSION¹

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INTRODUCTION

In a fall crop of Bonny Best greenhouse tomatoes at La Fayette, Ind., leaf mold caused by *Cladosporium fulvum* Cke. occurred in epiphytotic form in 1923 and produced a very prevalent and destructive, blackened, stem-end infection of the fruits of all ages. Opportunity was afforded to make a study of a large number of these infected fruits showing a wide range of symptoms.

The leaf-mold fungus has not been generally supposed to invade the fruit, although Plowright (11)³ in 1887 described and illustrated a fruit rot attributed to this fungus; Halsted (4) reported a rot resulting from inoculations with this fungus; and, according to Makemson (8, p. 317), Ferraris reported that it caused a scab disease of the young fruits. The symptoms observed in this outbreak at the Indiana station did not in any way resemble the scattered lesions described by Plowright or the scab type of lesion. In fact Plowright's description of stem stripes and his picture of the fruit lesions would indicate that the mosaic disease was present in the greenhouse from which he received the specimens. Nor is there any likelihood that this rot is related to that attributed by Plowright (10) and Smith (15) to *Cladosporium lycopersici*, inasmuch as that was a blossom-end-rot infection.

Makemson (8, p. 317), who made a study of this disease in Michigan greenhouses, found that all parts of the blossom, including the ovaries and young fruits up to the size of a pea, were infected. He reported blasting of the flowers by this fungus, and one case of a fruit one-fourth grown which bore conidiophores and spores. However he was unable to infect fruits by wound inoculation.

Other stem-end rots of tomato have been described. The stem-end rot reported by Dickson (1) does not resemble the *Cladosporium* rot in color and was caused by a *Botrytis* species. A dry, black, stem-end rot of green field-grown tomatoes in Virginia, resembling the one under consideration, but caused by *Phytophthora infestans*, was described and illustrated by Reed (12, p. 8) and by Fromme and Thomas (3, p. 8).

SYMPTOMS OF FRUIT INFECTION

The symptom which first attracted attention to this infection at the Indiana station was a sharply delimited jet black circular discoloration on the stem end of the young green fruits (pl. 1, A).

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² The writer wishes to acknowledge his indebtedness to Prof. H. S. Jackson for helpful suggestions.

³ Reference is made by number (italic) to "Literature cited," p. 539.

The surface of such fruits was smooth and unbroken, and at first there was no change in elevation (pl. 1, C). Later, the blackened areas tended to become more or less sunken (pl. 1, B). Soon a variety of manifestations of this peculiar stem-end rot was observed, the one constant feature being that it was always on the stem end of the fruit.

On the majority of the fruits the infected area extended rather irregularly away from the torus, usually farther on one side (pl. 1, E), and often the lesion seemed to be caused by more or less separate invasions radiating from the torus. This was very apparent in early stages of the invasion when a few small blackened areas were found extending out from the torus. In some cases a rather large lesion extended out only from one side of the torus, and on some fruits the discoloration first appeared as a slightly sunken collar or circular row of separate lesions centered around the stem end at a distance of $1\frac{1}{2}$ to 2 cm (pl. 1, D). Sixteen out of 210 fruits examined showed this type of infection. Ordinarily the lesion did not involve more than one-third of the surface of the fruit, and no fruits with surface totally blackened were found.

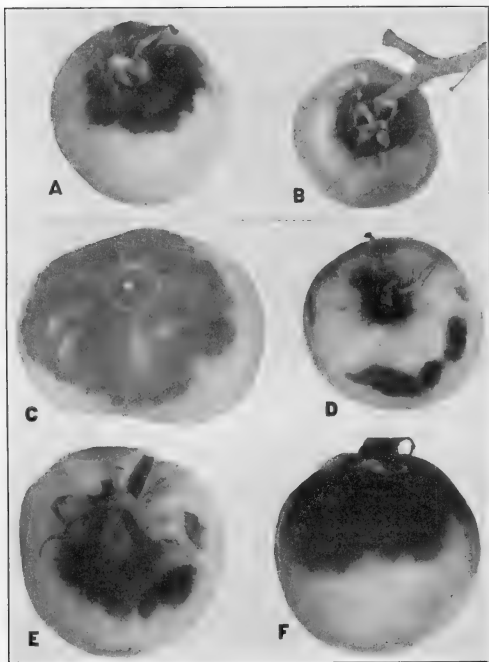
Owing to the radiating type of growth of the lesion, the margin tended to be irregularly lobed (pl. 1, A, C). Sometimes the margin was sharply defined, but frequently it was rather feathery (pl. 1, E) or the dark color blended rather gradually into the color of the apparently healthy part of the tomato (pl. 1, F).

The lesions were usually distinctly black, the color being more or less solid on the green fruits, while on red fruits it could often be seen that it was due to a blackened subepidermal network (pl. 3, A). In the former case the margin was either sharp or fading, in the latter case it was feathery or fringed. In addition to the black discoloration there were two other conspicuous color reactions. Very frequently rather small, whitish, papery patches, due to air under the epidermis, occurred on the blackened areas (pl. 2, C); and bright yellow sunken areas occurred occasionally on red fruits, probably owing to an inhibition of red pigment formation.

In the older lesions black raised subepidermal dots, very suggestive of pycnidia, occurred rather generally and were especially conspicuous in the whitish patches and yellow areas. Occasionally there were noticed on a fruit narrow, wedge-shaped, whitish or light green superficial strips radiating out from the torus, with their narrow ends toward the torus, and with scattered black dots on them.

Upon cutting longitudinally through the blackened stem-end lesions it was found that the entire thickness of the pericarp, as well as considerable portions of the locule walls and fleshy placental tissues, were involved in the blackening (pl. 2, D). Typically, there was no softening and very little structural disintegration of the affected tissues, but on the contrary these tissues were of a toughened spongy consistency. In some cases the blackened tissue could be torn loose and removed intact from the less coherent normal tissues. In other words, the affected tissues were somewhat mummified. Exceptions to this general condition were cases of secondary invasion of the killed tissues by bacteria.

Usually the internal blackening had penetrated somewhat further down into the placentae than in the pericarp or outer wall and was more extensive on the side showing the more external blackening.



A.—Jet black, stem-end discoloration of green tomato fruit, caused by *Cladosporium fulvum* Cke. Margin is sharply defined. Infection occurs through stomata in the sepal, torus, or pedicel, and the fungus eventually grows down into the fruit

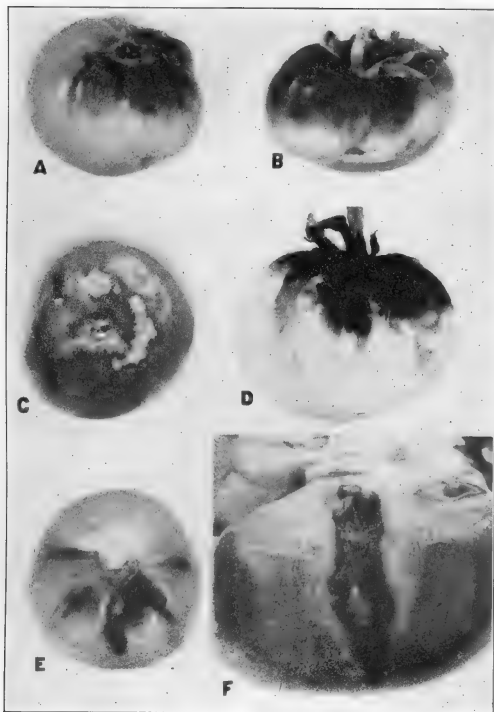
B.—Sunken, black, stem-end lesion on young tomato fruit

C.—Extensive blackening of the stem end of a green tomato. Margin sharply defined

D.—Collar of black lesions about the shoulder of the fruit more or less equidistant from the stem end. There is also some blackening about the torus. The mycelium can be traced back from such lesions to the torus

E.—Blackened lesion at one side of the stem end showing black dots under the epidermis and a feathery margin. The original infection probably occurred on one of the sepals on the side now showing the fruit discoloration

F.—Blackening of the stem end of a red fruit. The margin of the lesion is diffuse



A.—The type of asymmetry, or lopsidedness, produced by early infection of sepals and torus by *Cladosporium fulvum*. Radial furrows traverse the stunted side of the fruit, and the later invasion of the fruit by the fungus has produced a blackened area about the stem end on the stunted side.

B.—Lopsided fruit as a result of sepal infection. Radial furrows traverse the stunted side. The atrophy is due probably to the early presence of the fungus in that side of the torus. Later the fungus has grown down into the fruit, blackening the tissues.

C.—Stem end of an infected fruit showing whitish, papery lesions bearing stromatic or sclerotial bodies of the fungus. The white appearance is caused by air under the epidermis. The stromatic bodies may push through the epidermis in these papery spots and bear tufts of sporophores.

D.—Longitudinal section showing the blackening of the stem-end region invaded by the fungus. This fruit is slightly one-sided and the most extensive blackening is in the stunted side. Placental tissues and seeds, as well as pericarp, are involved.

E.—Blackened radial furrows emanating from the stem end, caused by *Cladosporium* invasion. One furrow shows a short transverse crack.

F.—Green tomato which showed very little external discoloration cut open to show an infected placenta. The basal part of the placenta and the young ovules were atrophied and killed and contained the abundant intercellular mycelium of the fungus. $\times 3$

Seeds in the affected region were dark brown in color. In both pericarp and placentae there was, in certain of the green fruits, some advance invasion of the blackening visible along the vascular bundles beyond the generally discolored region.

Dissection of the less blackened fruits, such as those showing a collar of black areas (pl. 1, D), disclosed the fact that the vascular bundles in the pericarp connecting these surface lesions with the torus were darkened. Upon removal of the torus it was found that usually there was a distinct darkening of the bundles in the scar, often more pronounced on the side showing the more extensive discoloration below.

There were other symptoms rather frequently associated with the disease in which the normal form of the fruit was considerably altered. One type of infection was represented by blackened furrows radiating from the stem end, in which the discoloration of the pericarp was rather deep seated (pl. 2, E). Short transverse cracks in these furrows were common, and it seemed evident that both the cracks and the furrows were attributable to a localized retardation of the growth of the fruit tissues. Dissection of such fruits showed that the dark color of the furrow was due to blackened tissue surrounding an underlying vascular bundle.

The affected fruits generally were somewhat asymmetrical or one-sided, the retarded side showing the most discoloration. This lopsidedness sometimes was very extreme, in which case the atrophied side was usually traversed by a few folds or furrows radiating from the torus toward the styler scar (pl. 2, A, B). Upon cutting certain of these fruits which showed rather slight external blackening, the startling discovery was made that the stem end of the placentae in some of the locules was brown and atrophied, and bore small dark bodies which were found to be stunted, infected ovules (pl. 2, F).

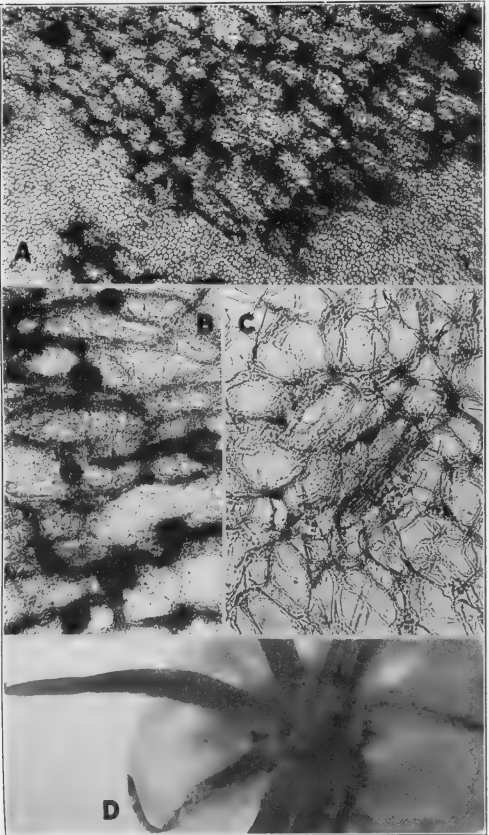
Once the significance of this lopsided condition of the fruits was appreciated, it was noted that many fruits of this character showed little or no discoloration, other than a tendency for the atrophied side to be yellowish rather than green or red. Many of such fruits developed the black color upon incubation, always more pronounced on the atrophied side, and other evidence was obtained (to be presented later) which indicated that this lopsided condition might be due to the retardation of growth on one side by this fungus under consideration.

It must be admitted, however, that this lopsidedness is not unlike that illustrated by Munson (9, p. 49) and attributed by him to incomplete pollination, an explanation which has been corroborated by Fink (2) and by later workers. It would seem, therefore, that the lopsidedness of tomatoes in the greenhouse may be due to more than one cause; in fact, mosaic necrosis and atrophy of a locule may also cause this condition.

Another striking symptom was the tenacity of the diseased fruits on the pedicels. Generally, the affected fruits were harder to remove from the plant than the normal fruits, and they did not fall off.

THE CAUSAL FUNGUS

An examination of unstained, free-hand sections of the darkened tissues revealed the presence of dense aggregates of coarse brown mycelium in the intercellular spaces (pl. 3, A, B, C; pl. 4). From the



(For explanatory legend see p. 525)

region of advance of the stem-end blackening in the interior of three small green fruits, portions of the tissue were cut out with a flamed scalpel and planted in poured plates of 2 per cent dextrose potato agar. From 22 of the 24 plantings a similar *Cladosporium* developed which, in cultural and spore characters, closely resembled the leaf-mold fungus, *Cladosporium fulvum* Cke. While the destructive effects of this fungus were very evident on the foliage of this crop, it was not suspected until these isolations were made that it was the cause of the fruit rot. The fungus was also isolated from the leaves by plates poured from a spore suspension, and it seemed to be culturally identical with the form isolated from the fruit.

To prove the identity of the cultures isolated from the fruit, five young tomato plants were sprayed with a spore suspension prepared from one of the cultures, and in 15 days typical yellowed leaf lesions appeared which, 5 days later, showed sporulation. Later a second series of five plants was similarly inoculated with spores from a culture isolated from another fruit; and leaf lesions appeared in 17 days, and sporulation 5 days later. The two series of control plants sprayed with water were not infected, and the disease was not observed to be present at any time on other plants in the greenhouse. These results prove beyond question that it was the leaf-mold fungus in the blackened fruit tissues. Some of the tissue plantings had yielded bacteria as well as the fungus, indicating that the latter may be closely followed by secondary invaders in the diseased tissues.

There was no sporulation of the fungus on the fruits, except in certain restricted locations, namely, about the margin of the torus scar, along radial epidermal cracks, and in the whitened papery spots already described (pl. 2, C). The sporophores were usually borne in dense tufts on rather conspicuous stromatic bodies, the black dots in the fruit lesions previously mentioned. In the papery spots, these stromatic bodies had burst through the epidermis and were densely covered with sporophores (pl. 4, B), but in general these bodies did not break through the fruit epidermis. Agar plates were poured from the spores produced on the torus scar on an infected fruit, and the colonies were identical with isolations from a leaf and from the interior fruit tissue.

Within the tissues of the fruit, the tendency of the fungus was to fill the intercellular spaces with composite strands of brown, thick-walled, geniculate mycelium, with short, swollen cells (pl. 3, B, C; pl. 4, D, E). The individual hyphae become undulating or geniculate, each eventually bearing numerous short lateral knobs or projections which dovetail and interlock with those of adjacent hyphae to form a pseu-

EXPLANATORY LEGEND FOR PLATE 3

A.—Surface view of epidermis over a fruit lesion (such as that in Pl. 1, E), showing the blackened subepidermal network produced by the mycelium between the large parenchyma cells under the epidermis, and a few scattered sclerotial or stromatic bodies—the black dots visible to the unaided eye. Photomicrograph $\times 41$. (All photomicrographs were made from unstained, free-hand sections of fresh material, unless otherwise stated)

B.—Tangential section of pericarp just underneath epidermis, showing the dense intercellular aggregates of brown mycelium and the spherical, intercellular, sclerotial bodies. Photomicrograph $\times 94$

C.—Section passing obliquely through a fibrovascular bundle in the pericarp tissue. Intercellular mycelium producing somewhat of a reticulum and forming denser aggregates between the parenchyma cells immediately surrounding the bundle, which is a characteristic behavior of the mycelium in the host tissues. Photomicrograph $\times 94$

D.—Calyx with one sepal (lower left) killed by early *Cladosporium* infection. The fruit was somewhat lopsided, the stunted side being under this sepal. Mycelium had penetrated into the fruit. Somewhat enlarged

doparenchyma. Makemson (8, p. 321) noted this type of stroma formation in his cultures on corn kernels, and it seems to be of common occurrence in culture.

In the larger intercellular spaces in the fruit tissue, not only immediately under the epidermis but throughout the interior tissues as well, this production of a pseudoparenchyma by the fungus reaches its maximum, and results in the formation of more or less spherical, stromatic, or sclerotial bodies just mentioned (pl. 3, B; pl. 4, D, E). Eventually these bodies become spherical and peritheciump-like, having an outer wall and oily contents, and measuring from 150 to 300 microns in diameter. Makemson (8, p. 323) noted the smaller stromatic bodies in the leaves, and found the larger bodies in his cultures on corn kernels. He (8, p. 320) describes the latter as "spherical bodies ranging from 50 to 120 microns in diameter" presenting "a structure typically perithecial in appearance with a thin, pseudoparenchymatous wall." These bodies, he states, were partially hollow but "contained neither asci nor spores, and could not be made to form spores by ordinary variations in the cultural technique."

In a culture on an autoclaved apple twig resting on moist cotton in the bottom of a test tube, these sclerotial bodies had developed in abundance on the cotton fibers at the end of 39 days. They had a dense dark brown wall and oily contents, and were accompanied by an abundance of short-celled, thick-walled, brown mycelium. These sclerotial or peritheciump-like bodies resemble in many ways the bulbils of certain fungi described and illustrated by Hotson (5). The formation of such bodies is apparently not uncommon among other *Cladosporium* species. Humphrey (6, p. 228) described hyphal knots formed by *Cladosporium cucumerinum* E. and A. in cucumber leaves, and Keitt (7, p. 13) found sclerotoid bodies formed by *C. carpophilum* Thum.

Cross sections of a tomato-leaf lesion near the midrib show that the fungus forms similar, but much smaller, mycelial clumps in the intercellular spaces of the mesophyll and in the substomatal chambers (pl. 4, A). Those in the latter position project or grow out through the stomata and bear tufts of sporophores, as Makemson (8, p. 323) has reported. A similar intercellular accumulation of mycelium occurs in the sepals, pedicels, and torus, in fact in all tissues in which the collapse and death of the host cells does not occur very soon after invasion.

EXPLANATORY LEGEND FOR PLATE 4

A.—Cross section of a leaf lesion of *Cladosporium fulvum*, showing dense intercellular aggregates of mycelium in the mesophyll and tufts of sporophores borne on mycelial aggregates or stromatic bodies in the stomata. The greater production of sporophores occurs on the lower epidermis. Photomicrograph $\times 110$

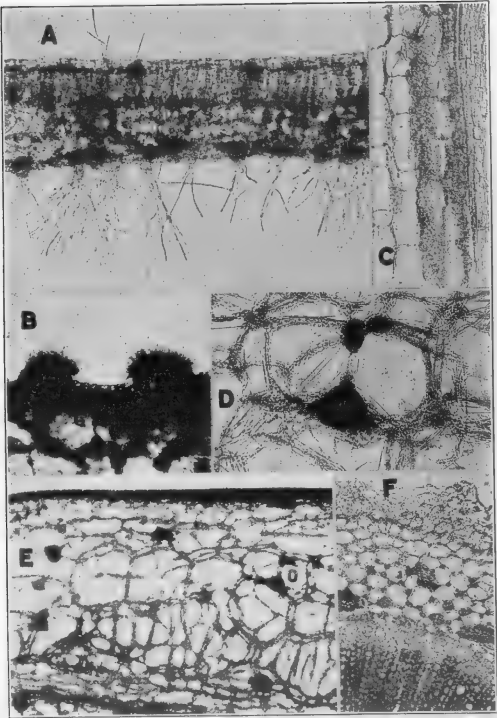
B.—Stromatic bodies covered with short sporophores. These bodies have pushed through the epidermis of the fruit in one of the white, papery lesions such as shown in Plate 2, C. In general the bodies remain subepidermal in the fruit lesions. Photomicrograph $\times 88$

C.—Longitudinal section through cortex of an infected pedicel showing dense aggregates of mycelium between the cortical parenchyma cells adjacent to the vascular tissue (phloem) at the right. Photomicrograph $\times 88$

D.—Enlarged view of the region marked O in the pericarp section in E, showing the multiple strands of mycelium between the cells and an intercellular sclerotial body 160 μ in diameter. Photomicrograph $\times 108$

E.—Cross section through outer portion of pericarp showing intercellular network or reticulum of composite mycelial strands. Tissue is so thoroughly infested that it is somewhat mummified, the cavities in the reticulum representing the original host cells. Scattered sclerotial bodies occur at various points, apparently in the larger intercellular spaces. The body at O is shown enlarged in D. Photomicrograph $\times 35$

F.—Cross section of an infected pedicel 3 mm. above the torus, showing dense masses of mycelium between the parenchyma cells of inner half of cortex, illustrating tendency of this fungus to accumulate near, but not within, the vascular tissues. Photomicrograph $\times 96$



(For explanatory legend see p. 526)

The ultimate function of these bodies has not been ascertained. Those that are exposed to the air serve as bases of sporophore tufts. They no doubt also constitute a resistant resting stage of the fungus. It was hoped that they might develop into perithecia, but no asci have been found. These bodies developed in abundance in a large number of infected fruits held about four weeks in a refrigerator, but all remained sterile. The infected stem regions were cut from these fruits, dried a month, and then incubated in sterilized, damp sand at temperatures of 10°, 18°, 22°, 27°, and 31° C. No ascus stage developed. However, the sclerotial bodies in the dried sepals and under the white papery spots on the fruit produced sporophores and spores in abundance. Therefore, it seems that these bodies may function as a resting stage resistant to desiccation and to other organisms. But it is not at all unlikely that other parts of the brown mycelial aggregates may function similarly.

The relation of temperature to the growth of the mycelium on potato dextrose agar was determined by inoculating the center of each of eight poured plates with a loopful of a spore suspension from a strain isolated from leaves, incubating these plates four days at 25° C., measuring the colonies, and then placing the plates in damp chambers in constant-temperature incubators. The increase in colony area based on the increase in average radius during 13 days was as follows: 0 sq. mm. at 2°, 8 at 10°, 25 at 16°, 55 at 20°, 52 at 24°, 21 at 27°, 23 at 30°, and 0 at 35° C. Sporulation occurred at all temperatures except 2° and 35° C. The optimum temperature for growth was 20° to 24°, exactly as Makemson (8, p. 328) found in his tests. He also found that 24° was the optimum for growth and sporulation in the living leaves. In a fruit held at room temperature, the black surface discoloration about the stem end increased 4 mm. in radius in three days. In general it may be said that the fungus grows rather slowly in culture.

The relation of temperature to spore germination was determined by placing the spores in a drop of sterile tap water or prune decoction on a flamed slide in a Petri dish damp chamber and incubating at temperatures of 10°, 16°, 20°, 25°, 30°, and 35° C. A high percentage (50 to 80 per cent) of germination occurred in water in 20 hours at 20°, 23°, and 30°, and a low percentage at 10°, 16°, and 35°. The germ tubes were short in all cases, but at 43 hours were very long at 20° and 25°. At 43 hours there was 80 per cent germination at 16°. Germination seemed as vigorous in water as in prune decoction. These results indicate that the optimum temperatures for spore germination represent a slightly wider range than do those for mycelial growth. Makemson (8, p. 329) found similar temperatures (18° and 24°) optimum for germination in hanging drops of water, and noted in infection experiments that long branched hyphae were formed after 36 hours.

RELATION OF FUNGUS TO HOST TISSUE

The location and distribution of the fungus in the host tissues was ascertained by examination of unstained free-hand sections of fresh material. The blackened discoloration of the fruit tissue was found to be due to the fact that the intercellular spaces were literally packed with dense aggregates of dark brown geniculate mycelium (pl. 3,

A, B). This mycelium so completely and thoroughly permeated the tissue that unstained free-hand sections gave the appearance, under the microscope, of a coarse reticulum of composite mycelial strands (pl. 4, E). This condition occurred throughout the blackened stem-end tissues, including pericarp, locule walls, and placentae. The mycelium was found considerably in advance of the margin of the darkened tissue, and here the intercellular strands were composed of fewer hyphae, more or less colorless, with longer cells of more uniform diameter.

The host tissues are not immediately killed by the intercellular mycelium, and in fact the tissues of certain organs such as the torus and pedicel and even young fruits remain alive many weeks after invasion. In fact, the fungus seems to establish a rather nicely balanced parasitic relationship with its host. In the fruits, however, the ultimate tendency of the fungus was to mummify the tissues by the formation of the heavy intercellular reticulum in which the lumina are the host cells. Thus the affected tissue gradually attains a tough, spongy character. The invaded tissues shrink or collapse very little because the original shape of the cells is preserved by the fungus reticulum.

The hyphal aggregates were always denser and larger in the large-celled parenchyma surrounding the vascular bundles (pl. 3, C; pl. 4, C, F), but apparently the mycelium does not invade the bundle tissues proper, possibly because of the comparatively small intercellular spaces. This tendency of the mycelium to accumulate in the parenchyma around the bundles accounts for the darkened bundles in the torus scar and radiating out through the pericarp and placental tissues. In its tissue invasion the fungus apparently progresses most rapidly along these particular intercellular channels. In leaf lesions resulting from inoculations, a blackened network due to this accumulation of the mycelium about the smaller veins was noted around the margin of the killed central tissues. Makemson (8, p. 323) also observed that the fungus in the leaves was most abundant around the tracheal tubes.

The black network noted in many of the fruit lesions was due to the hyphal aggregates between the larger parenchyma cells of the pericarp immediately under the epidermis (pl. 3, A, B). The dark radial furrows noted along the junctures of locule wall and pericarp in many fruits (pl. 2, E) were found to be due to similar aggregates of mycelium in the vicinity of underlying vascular bundles.

The fungus appears to be strictly intercellular (pl. 4, E). Hyphae were not found penetrating the host cells, even in the advanced stages of mummification of the tissue. In young fruits the growth of infected tissue and the tissues supplied by infected vascular bundles may be retarded or inhibited, and to this is attributed the furrows or depressions and the one-sided growth of the fruit. In cases of the latter showing no dark discoloration the mycelium was usually found present to a greater or less extent under the torus scar, particularly on the stunted side. In fact, in all types of fruit infection the mycelium was always to be found under the torus scar, and its distribution could be traced outward from that point. The brown, stunted, placental region at the stem end of certain fruits (pl. 2, F) was found to be entirely mummified by the intercellular aggregates of mycelium, and the young ovules were also invaded and killed.

Furthermore, as will be described later, the fungus invariably was found in the torus of infected fruits, a fact which may afford explanation for the observed failure of infected fruits to abscise normally. When infected fruits were picked, the torus usually remained firmly attached to the fruit and the break occurred at the pedicel node above the torus.

A study of a large number of cases of sepal, torus, and pedicel infection showed that the mycelium was intercellular in these organs, and, as in the fruit, formed dense aggregates in the parenchyma immediately adjacent to the fibro-vascular bundles, although none occurred within the bundles proper (pl. 4, C, F). The mycelium completely permeated the cortex, pith, and interfascicular parenchyma of the torus, but in the pedicel it was much more abundant in the cortex than in the pith. In general, the mycelial aggregates were not as dark as in the fruit and the dark stromatic bodies occurred less commonly. The distal portions of infected sepals were killed, but the tissues of the pedicel and torus apparently were not killed by the parasite, and infection of the latter organs could be detected only by microscopic examination for the presence of the mycelium, although tufts of sporophores were often present on large stromatic bodies in the stomata. Sporulation was abundant on infected sepals, especially on the lower surface, and the stromatic bodies bearing the sporophore tufts were well developed in the stomata of the sepals.

MODE OF FRUIT INFECTION

Halsted (4) reported successful wound infection with spores taken from leaves but did not prove the identity of the fungus in the fruit. He also reported one case of successful infection of the stem end of the fruit. Makemson (8, p. 322) proved that leaf infection by *Cladosporium fulvum* occurs through the stomata, but he was unable to obtain infection of fruits, wounded or unwounded. He observed infection of all parts of the flower, and states that fruits the size of a pea and smaller were unquestionably infected. This he explains (8, p. 319) on the basis that the young ovary possesses stomata and lenticels which may afford an entrance to the parasite and that these stomata are rapidly transformed into lenticels, so that by the time the fruit is as large as a pea no more infection can occur. But careful microscopic examination has revealed neither stomata nor lenticels in the epidermis of young tomato fruits and ovaries, although stomata have been found on the style, torus, sepals, and pedicel.

All attempts to produce infection by artificial inoculation of fruits in the laboratory gave negative or inconclusive results. Inoculation by puncturing fruits with a flamed scalpel dipped in a spore suspension, and by placing a drop of a spore suspension about the lower margin of the torus or on the stem-end scar from which the torus was freshly removed resulted in no infection.

The best clue to the mode of fruit infection was afforded by the distribution of the mycelium within the tissues. The outstanding feature of the disease was the constant association of the lesions with the stem end of the fruit. In the few cases among the several hundred fruits examined where the lesion did not visibly emanate from the stem-end scar, the mycelium could be traced back from the visible lesion along the fibro-vascular bundles of the pericarp to the stem

end by examination of free-hand sections. As previously stated, in all types of fruit symptoms the mycelium, if present, could at least be found under the torus scar, and could usually be traced outward from that region. This held true in entirety also for the encircling collar lesions and the dark furrows, while in the cases of the greenish or yellow area on one side and the asymmetrical, lopsided fruits the mycelium had not always grown very far from the scar.

Furthermore, it was discovered by study of free-hand razor sections that in all observed types of fruit symptoms the mycelium was invariably present in the torus, a fact which is considered highly significant. If infection occurred directly through some natural wound in the stem end of the fruit, such as a growth crack or the margin of the stem-end scar which is exposed as the enlarging fruit bursts the epidermal union between ovary and torus, it would not seem that the torus itself would invariably be invaded.

Not only was the torus infested with mycelium, but frequently certain of the sepals were invaded, and the mycelium was usually present in the pedicel as far as the first node above the torus (fig. 1, N) but not beyond that point. In a number of cases carefully studied, it was found that the infected sepal was directly above the point where the fruit invasion had progressed farthest from the point of stem attachment, or was above the yellowed or stunted side of the fruit. In certain cases the mycelium in the torus and pedicel occurred only in the side towards the infected sepal, or was more abundant in that side. In one case where there were two adjacent infected sepals, the most extensive development of the lesion on the fruit was on the same side.

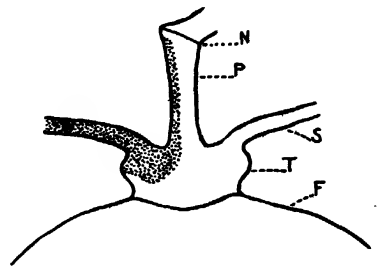


FIG. 1. — Diagrammatic longitudinal section of the pedicel node (N) and last internode (P), sepal bases (S), torus (T), and stem end of a young tomato fruit (F), showing by means of the shaded area the distribution of the mycelium of *Cladosporium fulvum* in one case observed.

In the case illustrated by Figure 1 none of the other sepals was infected, and this is regarded as an instance of sepal infection and subsequent mycelial growth down into the torus and up through the cortex of one side of the pedicel to the node. As a rule the torus eventually becomes completely permeated with the mycelium and the fruit tissue is invaded. The mycelium in the sepal or one side of the torus may retard the growth of that side of the fruit.

In one instance there were five darkened furrows in the fruit, and the five sepals above these furrows were infected, the central sepal most heavily, while the sixth sepal over the sound side of the fruit was not infected. In a case of an apparently normal fruit the mycelium in one sepal extended into the upper part of the torus cortex at the base of this sepal, and into the cortical parenchyma of the pedicel on that side, as shown in Figure 1. No mycelium was found in the fruit.

In another very similar case the mycelium extended to the base of the torus, and upon removal of the latter a slight darkening was visible about the bundles in the scar on the fruit. This fruit was incubated at room temperature, and in 10 days an infected zone 6 mm. broad was visible about the scar. In a third very similar case an infected zone 7 mm. broad appeared in 10 days, accompanied by a few black subepidermal stripes radiating from the torus scar.

These observations indicated that beyond doubt there was a correlation between the stem-end fruit invasion and the presence of the mycelium in the sepals, torus, and pedicel, and that the latter condition bore a causative relation to the former. The presence of stomata in the sepals, torus, and pedicel would readily permit spore infection, and it would seem very likely in the cases just cited that infection had occurred in the sepal, the mycelium proceeding from the sepal down into the torus and thence up into the pedicel and down into the fruit. In other cases examined no mycelium was seen in the sepals, indicating that spore infection probably took place on the torus or pedicel, both of which bear stomata. Apparently the mycelium can not pass the pedicel node (fig. 1, N), so infection, to reach the fruit, probably must occur below the first pedicel node above the torus.

Fourteen of the lopsided fruits, including some remaining green or yellow on the atrophied side and some showing the color retardation without any marked asymmetry of shape, were searched for mycelium, and in all but three cases a little was found directly under the stem-end scar. The torus was present on six, and in every case it contained mycelium. In freshly picked specimens, the mycelium was found in the atrophied or yellowed side of the fruit, and ordinarily was scanty, seldom permeating any considerable proportion of the affected side. Therefore, it would seem very likely that the lopsidedness and yellowing of such fruits was attributable to the early presence of the mycelium in the torus or pedicel, the region through which the water and food materials for the fruit must pass. However, the fungus does not seem to kill the tissues in the torus and pedicel.

These observations suggested that fruit invasion was probably the result of very early spore infection of the sepals, pedicel, or torus. To test this theory, a number of inflorescences were inoculated, in the greenhouse in March, by placing a loopful of a spore suspension on the uninjured pedicel, torus, sepals, or stigma. In practically no cases had the ovary more than barely begun to enlarge, and, in the cases of pedicel, torus, and sepal inoculation, these organs were first sprayed with distilled water before the spore suspension was applied. In 9 out of 37 cases of sepal inoculation, and 2 out of 6 cases of blossom inoculation on the last internode of the pedicel, the fungus was found sporulating on the sepals within three to five weeks.

An examination of razor sections of the stem end of the fruit 8 to 10 weeks after inoculation showed the typical intercellular *Cladosporium* mycelium in 5 of the 37 cases of sepal inoculation, 1 of the 4 cases of torus inoculation, and in 2 of the 6 cases in which the last internode of the pedicel was inoculated. No invasion occurred in the 4 cases in which the next to the last internode of the pedicel was inoculated, nor in the 5 cases of stigma inoculation. The identity of the mycelium within the fruits was proved by tissue transfers to agar. It was rather surprising to find certain fruits completely infested with the intercellular mycelium and yet showing no conspicuous discoloration of the tissues. In one case of sepal inoculation a typical, dark, stem-end rot of the fruit was noted 14 weeks after inoculation. These tests prove that fruit invasion may result from very early spore infection of the sepals, torus, or last pedicel internode.

In a number of the cases of sepal inoculation the fruit became lop-sided, with the atrophied side usually longitudinally furrowed and slightly yellowish. In four cases the atrophied side was under the sepals which first exhibited *Cladosporium* sporulation and which were most likely the particular sepals originally infected as a result of the inoculation. In one case the fungus was sporulating on three adjacent sepals, and the side of the fruit under these sepals was atrophied and was traversed by a longitudinal furrow under each of the three sepals. Upon sectioning, however, no mycelium was found in the fruit nor the torus but was found only in these three sepals just mentioned. Somewhat similar cases have herein been described previously in connection with torus infection and lopsided fruits. Therefore it seems probable that the presence of the fungus in the sepal may have an injurious effect upon the corresponding sector of the fruit.

In six cases the mycelium was found in the cortical tissues of the last internode of the pedicel (fig. 1, P), but never above the first node above the torus (fig. 1, N), a condition previously noted in connection with pedicel infection. This indicates that the mycelium is unable to pass through this node. As the fungus is strictly intercellular and readily invades the fruit from the torus, it seems likely that the intercellular spaces are continuous throughout the sepals, last pedicel internode, torus, and fruit; while the failure of the fungus to pass upward beyond the first pedicel node would seem to indicate that the intercellular spaces were not continuous through that node. Owing to the absence of stomata in the fruit, the continuity of the intercellulars of the sepals and the fruit tissue may be of some physiological significance.

Observations made in a greenhouse crop in August, 1924, about the time the lowest clusters of fruit were mature, gave further evidence that fruit invasion results from sepal infection. Leaf mold was extremely severe on the foliage; and while no black stem-end rot was found, very abundant sepal infection (pl. 3, D) had occurred as was evidenced by the sporulation of the fungus on the sepals. The prevalence of sepal infection was determined by an examination of a large number of calyces in the lower five or six clusters. (See Table I.)

TABLE I.—Prevalence of calyx infection in greenhouse tomatoes

	Number calyces examined	Total per cent infected	Percentage infected on—					
			1 se- pal	2 se- pals	3 se- pals	4 se- pals	5 se- pals	6 se- pals
Ovaries less than 1 cm. diameter.....	243	52	34	12	4	1	0.4	-----
Fruits more than 1 cm. diameter.....	294	80	36	20	16	5	3	0.3

A very high percentage of the calyces were visibly infected, even in the case of small ovaries, and in many instances more than one sepal was infected. In view of the two to three weeks of incubation between infection and sporulation, it would appear that much of this infection had occurred during the blossoming period; and it probably is this early infection which is most likely to give the fungus time to grow down into the fruit before the latter ripens and is picked.

Under the microscope, longitudinal sections of some of the ovaries with infected sepals showed the mycelium present in the sepal base and pith of the pedicel but not within the ovary. In one case of a green fruit with an infected sepal the mycelium was traced from the edge of the sepal lesion down within the tissues of the sepal into the torus, a distance of 10 mm. In the case of one lopsided green fruit, one sepal was already killed by the fungus (pl. 3, D), and it was located directly above the stunted side of the fruit. There was no discoloration of this fruit to indicate infection, but microscopic examination revealed an abundance of *Cladosporium* mycelium in the fruit tissue at the stem end.

The blackened stem-end discoloration is a rather uncommon effect of this fungus in greenhouses, which may be due to the fact that ordinarily the fruits are picked before the fungus has had time to grow from the sepals into the fruit; or, if the fungus has already penetrated into the fruit, the fruit is disposed of before the conspicuous symptoms develop. The particular crop in which the fruit symptoms were so conspicuous had been very much retarded by cloudy weather.

SEED INFECTION

The advance of the mycelium of *Cladosporium fulvum* from the stem end down through the pericarp of the fruit is paralleled by its penetration into the axile placentae (pl. 2, D). In some cases the ovules are infected and killed when very young. In cases of extreme atrophy of a locule in the lopsided fruits the ovules may never develop into seeds, even though not actually invaded by the fungus. Ordinarily, however, extensive invasion of the fruit occurs at a later stage after the seeds have developed, and the placental tissues are blackened and mummified by the abundant intercellular mycelium, while the seeds in the invaded region become darkened and many show a distinct blackening of the hilum end (pl. 5, A).

Free-hand sections of the placental tissue showed the typical mycelial aggregates between the parenchyma cells around the fibrovascular bundle extending out into the funiculus of the seed. Longitudinal sections of a seed with a blackened hilum showed that the mycelium had penetrated well within the outer integument

EXPLANATORY LEGEND FOR PLATE 5

A.—At left: Four tomato seeds removed from the invaded stem-end region of a fruit. These are darkened and show blackening of the hilum. At right: Four noninfected seeds from same fruit. Enlarged $\times 1\frac{1}{2}$.

B.—A tomato seed infected at the hilum. The seed was incubated on moist filter paper in a damp chamber. The blackened hilum is covered with sporophores and spores of *Cladosporium fulvum*. Potted plants were infected successfully with spores from such a source. $\times 12$.

C.—Seed coat, with blackened, infected hilum, carried up on cotyledons of seedling. The fungus was producing spores on the blackened tissue in this case. Enlarged $\times 8$. In lower right corner: Primary infection of cotyledon from seedling grown from an infected seed such as shown in B. The cotyledon is covered with sporophores and spores of *Cladosporium fulvum*. Enlarged $\times 3$.

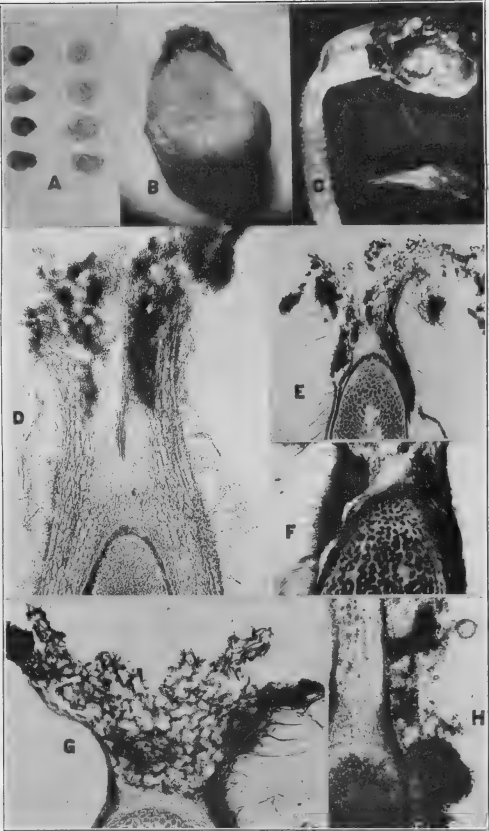
D.—Longitudinal section of the hilum end of an infected seed, showing dense aggregates of brown mycelium in parenchyma tissue of middle layer of the perisperm or seed coat. The spiral vessels of the fibrovascular bundle are shown. Part of the endosperm is seen at bottom. Photomicrograph $\times 96$.

E.—Longitudinal section of hilum end of an infected seed, showing mycelial aggregates or peritheciump-like bodies in the funicular tissue remaining attached to the seed and also crowded between the outer integument and endosperm. With collapse of the parenchyma of the middle layer of seed coat, these bodies cause the outer integument to bulge outward. No invasion of the endosperm has been noted. (Stained with carbol fuchsin). Photomicrograph $\times 36$.

F.—Mycelial aggregates crowded between outer integument and endosperm. (From a longitudinal section of same seed shown in E, stained in carbol fuchsin.) Photomicrograph $\times 72$.

G.—Longitudinal section of hilum end of infected seed, showing the intercellular mycelium in parenchyma of middle layer of the seed coat extending down almost to the endosperm, the edge of which is seen at the bottom. One peritheciump-like or sclerotial body is shown at the left. (Triple stain.) Photomicrograph $\times 88$.

H.—Mycelial aggregates attached to exterior of seed at hilum end by entanglement of the mycelium with the cellulose rods or hairs of the seed coat. Photomicrograph $\times 81$.



(For explanatory legend see p. 534)

at the hilum and had formed the usual dark-brown intercellular aggregates of mycelium in that part of the parenchyma of the middle layer of the seed coat which remains uncollapsed in the hilum region of the seed (pl. 5, D, G). These mycelial aggregates were conspicuous immediately around the fibrovascular bundle which extends from the funiculus into this tissue. Sclerotial bodies were also formed in this tissue (pl. 5, E, F, G). In certain instances observed the mycelium had invaded the parenchyma of the middle layer as far as the boundary of the endosperm (pl. 5, G), and in some cases had even grown down a short distance at the sides of the endosperm, forming dense aggregates and occasionally sclerotial bodies in this region. With the collapse of the middle layer, these bodies are crowded between the endosperm and the outer seed coat and cause the latter to bulge outward at that point (pl. 5, E, F). No mycelium was detected within the endosperm, however, probably because of the apparent absence of intercellular spaces.

In addition to the internal infection of the seeds, there was also a more or less extensive mycelial infestation of the palisade layer of long cellulose rods or hairs which cover the outer integument of the seed. The mycelium in some cases had accumulated in these hairs near the hilum and formed well developed sclerotial or peritheciump-like bodies entangled among the hairs and thus firmly attached to the seed coat (pl. 5, H). Thus it seems that the seed may be infested by the fungus externally as well as internally.

The identity of the fungus within and on the seeds was proved by incubating the latter in a moist chamber. Eleven seeds which showed infected hila and which had been dried 10 days were placed on moist filter paper in a Petri dish. Abundant sporulation of *Cladosporium* occurred on the blackened tissues of 6 of these seeds within 8 to 13 days (pl. 5, B). Long spore chains were produced, some containing as many as five spores. The spores were typical of *C. fulvum* and prompt germination occurred in water. In fact, many of the spores had germinated in the damp air of the Petri dish. Agar plates poured from a suspension of these spores yielded typical *C. fulvum* colonies.

Leaf infection of young tomato plants was obtained by spraying with a suspension of the spores from one of the cultures isolated from a seed. Sporulating lesions were found 23 days afterwards on all of the inoculated plants, while the check plants remained healthy. One plant was successfully inoculated by spraying the lower sides of the leaves with water and touching some of the drops with a seed coat bearing conidia about the hilum.

In one of the cases of successful sepal inoculation previously described, the fruit was examined 14 weeks after the inoculation and it was found that abundant seed infection had occurred.

SEED TRANSMISSION

That the mycelium of *Cladosporium fulvum* may invade the seed and establish itself on and within the seed coat has been shown. In no case does it appear that the endosperm or embryo is actually invaded by the fungus. The parasite may also gain access to the seed in another way. It was found that in extracting the seed from diseased fruits fragments of the blackened, infected fruit tissue very frequently adhere to the surface of the seed, and that, after

drying, these fragments were not easily dislodged. Likewise, *Cladosporium* spores from infected sepals might lodge on the surface of the seed. The likelihood of seedling infection from the seed-borne fungus depends upon a number of factors, some of which have been studied.

The viability of the fungus in and on the seeds has been proved and its behavior determined by incubating the seeds on moist filter paper in damp chambers. Sporulation occurred on both the actually infected and the contaminated seeds. In some cases spores formed in abundance after four days at room temperature. Eleven seeds with infected hila were incubated at 27° C., and sporulation occurred on 6 at the end of 13 days. Thirty seeds bearing blackened tissue fragments were similarly incubated, and sporulation occurred on the blackened fragments of 3 of them after 13 days. Many of the spores germinated in the saturated atmosphere of the damp chamber.

With regard to the longevity of the fungus in and on the seeds, it should be noted that the seeds in the two tests just mentioned had been dried only 10 days, while later tests proved the fungus alive after one month. It would seem that the dense mycelial aggregates within and upon the seed coat were excellent adaptations for the survival of long periods of desiccation. Savelli (13) and later Makemson (8, p. 335) found that the spores endured desiccation for six months and the latter found that some spores a year old were viable.

It is important to note that the moisture and temperature conditions conducive to germination of the seed are likewise conducive to sporulation as well as to spore germination, particularly if the seed lies upon the surface of the soil or if the seed coat is carried up by the cotyledons, a very common occurrence among tomato seedlings. It is to the advantage of the parasite that the infected or contaminated seed should germinate and carry the seed coat up above ground.

The germinability of the seeds bearing tissue fragments obviously will be unharmed. Of the 30 seeds of this type tested on moist filter papers as already mentioned, 27 germinated and 14 carried the testa up on the cotyledons. In one of these latter cases the fungus was sporulating on the tissue fragment. Of the seedlings grown in sand or greenhouse soil, however, a lower percentage carry up the seed coat. Of the 11 seeds with infected hila which were similarly tested, 8 germinated and one carried up its seed coat (pl. 5, C), in which case the fungus was found sporulating on the infected tissue of the hilum. In another test, 9 out of 10 infected seeds germinated. Thus, apparently, the invasion of the seeds by *Cladosporium fulvum* does not materially reduce their germinability, and such seeds, as well as those bearing infected tissue fragments, will germinate and in certain cases carry the seed coat up into the air.

The tests just noted show that, by means of infected or contaminated seeds, the parasite might be introduced into the seed bed and be carried above ground in a sporulating condition and that spore infection of near-by seedlings might very easily occur, whether the offending seedlings were themselves infected or not.

However, in the case of hilum infection, there is ample opportunity for the seedling itself to become infected, because both radicle and cotyledons emerge from the seed coat directly through the infected region (pl. 5, C). Observation of a specific instance where a radicle, in contact with an abundance of the spores and mycelium

remained uninfected, indicates that this organ is immune to infection, probably because of its lack of stomata. But the curved hypocotyl emerging from the same opening in the end of the seed coat drags after it the two cotyledons which must slide through in direct contact with the infected tissue, thus exposing them to spore contamination (pl. 5, C).

When wetted, the spore chains on the sporophores break up at once, the spores being readily dispersed in a film of water such as is very likely to be continuous from the seed coat to the emerging cotyledons, at least at some time during their emergence. Examination of an empty seed coat showed that the fungus was sporulating along both edges of the opening through which the cotyledons had emerged.

Since in the tests just noted the seedlings could not be grown in the damp chambers long enough to obtain evidence of infection, similar plantings were made, February 7, in pots of steamed sand placed in an inclosed compartment in a greenhouse in which the disease had never been present. These pots were watered with such care that there was practically no spattering. Not very many of these seedlings carried the seed coats up into the air. An examination made after 29 days revealed no infection, but 10 days after that 2 seedlings, each of which bore an infected cotyledon, were found (pl. 5, C). One of these was among the 9 seedlings grown from seeds with infected hila, and 1 among the 40 seedlings grown from seeds bearing adherent fragments of the infected fruit tissue. These seeds had been dried one month before planting. In these cases of primary infection there was abundant sporulation of the parasite on both surfaces of the infected cotyledon, more abundant however, on the lower. From such primary infections, the spores might readily be spread by air and water to other plants in the plant bed. Thus the disease might establish itself in a crop by primary infection of the cotyledons from either infected or contaminated seeds.

It seems then that the disease may be transmitted by infected or surface-contaminated seed as a result of the sporulation of the fungus on the infected tissues. If there is any fruit infection in the fields from which commercial seed is saved, the disease may be widely disseminated through such seed. The disease occurs only to a slight extent in the field crop in Indiana; it seems to be more prevalent as a field disease in more humid regions. It has been reported as a serious field disease near Norfolk, Va., by Smith and Zimmerley (14); and it has been reported from South Carolina; and, according to the reports of the Federal plant disease survey, it has occurred as a field disease in Florida, Louisiana, Virginia, Kentucky, and Ohio. It would seem possible, therefore, that fruit infection may occur in seed fields. Greenhouse growers who are saving seed should guard against the selection of infected fruits.

SUMMARY

A conspicuous, black, stem-end rot of both immature and ripe greenhouse tomatoes has been found to be caused by the leaf-mold fungus, *Cladosporium fulvum* Cke.

This fungus also causes blackened radial furrows in the fruit, and lopsided fruits which tend to remain green or yellow on the retarded side.

The mycelium is intercellular, and the fungus produces composite strands or pseudoparenchymatous aggregates which mummify the host tissues and form a reticulum in which the cavities represent the original host cells. The mycelium is not found within the fibrovascular bundles, but completely permeates the parenchyma and tends to accumulate most extensively between the parenchyma cells immediately adjacent to the vascular bundles. Invaded tissues may remain alive for a considerable period.

Scattered throughout the affected tissues, the fungus forms, in the larger intercellular spaces, sclerotial or peritheciump-like bodies or bulbils 150 to 300 microns in diameter. No spores have been found within these bodies. Those formed under stomata in sepals, pedicel, and torus, and in certain restricted areas on the fruit lesions, may bear tufts of conidiophores.

Mycelial growth and spore germination occur between 10° and 30°, with an optimum at 20° to 25° C.

In an infected fruit the fungus occurs in the pericarp, locule walls, and placentae, invariably, it would seem, in the torus, and frequently in one or more sepals and the last internode of the pedicel.

No stomata have been found in the fruit. Histological studies and inoculation tests indicate that spore infection occurs rather early through stomata in the sepals, torus, or last pedicel internode, after which the mycelium grows down into the fruit, causing dark discoloration. The presence of infection in the sepals or torus may produce a stunting effect on one side of the fruit which results in lopsidedness of the fruit. Sepal infection is of common occurrence.

The mycelium grows down through the placentae and invades the seeds both externally and internally. Sclerotial bodies are attached to the exterior of the hilum end of the seed coat. The fungus also grows into the parenchyma tissue of the middle layer of the seed coat at the hilum, penetrating as far as the endosperm, but not into the latter. Sclerotial bodies are formed within this middle-layer tissue.

Fragments of infected fruit tissue may adhere to the seed coat of seeds extracted from diseased fruits.

Under moist conditions the fungus sporulates on infected and contaminated seeds. Since the seed coat is often carried up on the cotyledons of the seedling, the spores may readily reach other seedlings.

In germination, the cotyledons must emerge through the infected hilum region. Primary cotyledon infection occurred among seedlings grown in pots of sterile sand from both infected and surface-contaminated seed which had been dried one month.

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FLUCTUATION IN THE DISTRIBUTION OF THE COLORADO POTATO BEETLE¹

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INTRODUCTION

Fluctuation in the abundance and distribution of the Colorado potato beetle (*Leptinotarsa decemlineata*) is a constantly observable phenomenon in a potato-growing region. So inconspicuous does this insect become during occasional years that some growers do not feel justified in using a poison with their early sprays. Furthermore, in any year the distribution of the species in a given locality is extremely uneven. Great variation is found in the percentage of infestation from field to field, from farm to farm, and from one locality to another, in the same year as well as over a period of years. No satisfactory explanation of the phenomena has been offered except that of parasitism. Field studies, however, reveal certain variable factors operating in a northern latitude to effect variation in the abundance of the Colorado potato beetle in adjacent fields and also an important factor which influences the abundance of the insect over a series of years. It may be desirable to set down evidence of the nature of these variables as well as certain conclusions that may be considered with profit by growers.

FIELD STUDIES

The operation of these variables was demonstrated in field studies undertaken to determine whether correlation existed between the percentage of infestation and various environmental factors. The data in this study, involving six varieties, were taken during the period August 17 to 23, 1919, a year of comparatively light infestation, in 12 townships of northern Maine, from 57 potato fields with a total of 786 acres. In contrast, data are used that were taken from the same fields, with a few exceptions, in a year of comparatively heavy infestation, during the interval August 23 to 25, 1921, from 27 fields totaling 273 acres. During the interval September 8 to 11, 1924, similar studies were undertaken in 3 counties in western New York in 28 fields totaling 226 acres. The conclusions derived from these studies have been confirmed by field observations in other northern potato-producing regions. The data taken at all fields being the same, included the following: Variety, acreage, date of planting, type of soil, fertilizer, windage, slope, sprays, disease, condition of the crop, and the percentage of the plants infested. The percentage of infestation figure was determined from field readings of 100 plants taken at random throughout the field, and was based on the presence or

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absence of foliage injuries. The figure indicates, but does not represent, the degree of severity of the infestation. Tabulation and comparison of these readings indicate that the only variables affecting the percentage of infestation are earliness of appearance of the potato shoot above ground, type of ridge cultivation (covering operation), the nature of the previous crop, the application of the early sprays, and the proximity of wild or "volunteer" potato plants.

PROTECTION OF EARLY COVERING

The "ridge" rather than the "hill" method of cultivation is followed consistently throughout the district in northern Maine from which figures were obtained. After breaking up the soil with a 4-inch cultivation, a disk hoe (occasionally a blade hoe) is used to throw the loose soil into a somewhat sharp ridge. Naturally, a deeper cultivation results in higher ridges. Ridging is begun when the potato sprouts first break through the surface soil. The sprouts are covered to a depth of 2 or more inches. The practice is then repeated as soon as the sprouts appear again, and sometimes they are covered for a third time before the rate of growth becomes too rapid to keep the sprouts covered. Ridge cultivation confers several advantages in addition to protecting the young plants from infestation. Great variation occurs in the height as well as the care with which the ridge is constructed during the covering operation as well as subsequently. In this paper it is indicated whether a low, medium, or high ridging was practiced. In general, high-ridge cultivation is correlated with more compact covering. An indication of the protection afforded by the covering operation may be seen in Table I, which gives data taken from adjacent fields where, other conditions remaining the same, both low and high ridging were practiced side by side.

TABLE I.—High-ridge versus low-ridge cultivation

A.—IN TYPICAL AND ADJOINING FIELDS						
Field No.	Variety	Planted	Fertilizer	Previous crop	Ridging	Per cent infested
136	Bliss Triumph.....	May 21	4-8-4	Potatoes.....	High.....	20
137do.....	May 18	4-8-4do.....	Low.....	100

B.—AS INDICATED IN ALL FIELDS STUDIED								
Type of ridging	1919				1921			
	Number of varieties	Number of fields	Acreage	Per cent infested	Number of varieties	Number of fields	Acreage	Per cent infested
Low ridging.....	4	7	35	82.57	3	9	60	97.22
Medium ridging.....	6	27	445	36.66	4	10	50	84.70
High ridging.....	6	23	206	23.08	3	8	186	63.62

Ridging is not uniformly the practice in western New York, and where this method is followed it is not always done early enough to cover the shoots. Here growers cover more often for weed eradi-

cation than for insect control. Planting dates are later, and hence no protection from frost injury is conferred. It is evident, furthermore, that both the ridging and the covering operations under western New York conditions are done as the season dictates and are not standard cultivation practices. That the covering operation had a protective value during the season of 1924 in western New York is shown in Table II.

TABLE II.—Control effect of the covering operation in western New York

Operation	Not covered	Covered
Number of varieties.....	5	6
Number of fields.....	16	11
Acreage.....	143	82.9
Per cent infested.....	50.91	13.34

INFLUENCE OF THE PREVIOUS CROP

Examination of field data also indicates that the nature of the previous crop has a marked influence on the infestation of the Colorado potato beetle. The usual rotation practice followed in the regions studied places sod a year ahead of potatoes, but when potatoes precede potatoes in the same soil a very considerable increase in the percentage of infestation has been noted. In respect to this point, the writer's figures confirm a relation already observed by keen growers. The fact that heavier infestation occurs in fields planted the second year to potatoes may be explained by the natural sluggishness of the overwintering adults on emergence in the spring. Finding the cultivated potato close at hand they accept the plant and give rise to the first brood upon it. The origin of these overwintering adults may be traced to two sources. Injured tubers left in the field after harvest attract late-emerging adults of the second brood which are obliged to accept them as food in place of foliage for a short interval before entering winter hibernation quarters. Naturally the presence of overwintering adults in soil previously planted to potatoes is also explained by infestation the previous year. The importance of this factor of the previous crop in influencing the percentage of infestation is shown in a comparison of two adjacent fields in which potatoes were growing under identical conditions, except that in one the previous crop had been potatoes and the other had been in sod (Table III).

TABLE III.—The effect of the previous crop

A.—IN TYPICAL AND ADJOINING FIELDS

Field No.	Variety	Planted	Fertilizer	Ridging	Previous crop	Per cent infested
2291	Jersey Giants.....	May 10	3-8-6	Medium.....	Potatoes.....	87
2292	do.....	do.....	3-8-6	do.....	Sod.....	2
2176	Irish Cobbler.....	do.....	4-8-4	do.....	Potatoes.....	28
2177	do.....	May 5	4-8-4	do.....	Sod.....	2

TABLE III.—*The effect of the previous crop*—Continued

B.—AS INDICATED IN ALL FIELDS CONSIDERED IN NORTHERN MAINE

Previous crop	1919				1921			
	Number of varieties	Number of fields	Acreage	Per cent infested	Number of varieties	Number of fields	Acreage	Per cent infested
Potatoes.....	4	26	259	56.07	3	11	61	95.45
Sod.....	7	31	427	24.51	4	16	212	73.81

C.—AS INDICATED IN ALL FIELDS CONSIDERED IN WESTERN NEW YORK

Previous crop	Number of varieties	Number of fields	Acreage	Per cent infested
Potatoes.....	4	5	46.75	76.2
Sod.....	6	23	179.4	32.8

Under western New York conditions the same relation holds true (Part C, Table III).

EARLINESS

A third variable factor influencing the percentage of infestation is the earliness with which the plants make their appearance above ground. This is usually thought of in terms of planting date, or variety, or fertility of the soil, but in any case the result is the same. If a field is planted to an early variety such as Irish Cobbler, or if a late variety is planted early or in a favorable situation where it receives a stimulus to early and rapid growth, the percentage of infestation will be very much greater. The potatoes which come up earliest are infested first, and natural dispersal to varieties appearing later is slow and usually inconsiderable except where influenced by spraying. An indication of how this factor operates in adjacent fields in which all factors remain the same except the earliness with which the plants push through the top soil is shown in Table IV.

TABLE IV.—*The influence of variety*

TYPICAL ADJOINING FIELDS OF SAME VARIETY PLANTED EARLY AND LATE

Field No.	Variety	Acreage	Fertilizer	Ridging	Previous crop	Planted	Per cent infested
1927	{Spaulding.....	30	4-8-6	High.....	Sod.....	May 5	40
	{Rose.....						
1933	{Spaulding.....	5	4-8-2	Medium.....	Sod.....	May 20	20
	{Rose.....						
1928	{Bliss.....	20	4-8-6	High.....	Sod.....	June 10	14
	{Triumph.....						
1930	Irish Cobbler.....	30	4-8-6	High.....	Sod.....	May 10	65

Colorado potato beetles do not select early varieties exclusively, neither do they shift from late to early variety habitually. A late variety planted early will “trap-crop” for early varieties planted late, as may be noted by comparing the results in the adjoining fields Nos. 1927, 1928, and 1930.

Apparently the adult insects drop into a potato field without deliberation and are satisfied if they find potato plants. Observations made in the Solanaceae garden at Ithaca in 1923 indicate that distinction is made within the family. A common idea is current among growers that Colorado potato beetle adults manifest a preference for early varieties when offered a choice of host plants, but it would appear from variety tests that the insect does not have any particular preference when offered the opportunity of wide choice of varieties. It would seem that the insect arrives on some varieties only after trial and error, and that it does not make a distinction between varieties. This has been indicated in variety-test plots where all factors remained the same except variety.

THE VOLUNTEER

That early spraying of potato sprouts soon after they come up is effective in reducing infestation, is a common observation among growers and in the literature of the subject. In addition to the lethal effect of the spray there is a certain amount of protection afforded by the deterrent influence of the Bordeaux-arsenical sprays. Redispersal flights often closely follow spray applications. Repelled from further feeding by either the spray residues or alimentary disturbances occasioned by arsenic ingestion, the beetles take flight for a more hospitable environment. One may locate them sometimes resting on nearby foliage, as if contemplating what to do next. Such redispersal flights afford an opportunity for infestation of other fields and wild Solanaceae, the most common of which is the wild or "volunteer" potato.

The "volunteer" potato is a plant which has grown from tubers remaining overwinter in the ground or from tubers thrown away in waste areas. In a northern latitude they occur most frequently in fields sown to oats, wheat, and other similar grain crops following potatoes. Low temperatures prevent decay in the fall, an early snow blanket protects from heavy ground frost, and tubers missed during the previous fall harvest develop plants the following spring which come up with the new crop. It is not unusual for the plant to perpetuate itself; volunteers may be found in the third and sometimes the fourth year after the field was originally planted in potatoes. Great fluctuation in the numbers of volunteers per acre occurs, due to the fact that in some years the ground freezes deeply before the snow comes and the tubers are killed and "volunteers" are either very scarce or absent the following year. Dispersing adult beetles are then forced to choose between wild species of *Solanum* and the cultivated crop. After a deliberate hunt for infested species of wild Solanaceae, the writer is convinced that the beetles do not accept them within smelling distance of cultivated potatoes. This opinion, based on observation, is confirmed by the fact that high rates of infestation follow years favorable to the development of volunteers, such as were the years in which the data reported in this paper were taken. The importance of the volunteer potato plant as a refuge and reservoir for the potato beetle is not overdrawn. In one locality the writer counted 202 volunteers per acre in wheat, with an average infestation of 36 per cent; in wheat in another locality they ran 3,360 per acre, with an average infestation of 16 per cent, and in clover 621, with 54 per cent infestation.

The volunteers in a hay or grain field often are quite sufficient in numbers, therefore, to attract dispersing beetles, but they are not always the most important source of infestation. In grain fields where cultivation has broken up the soil, volunteers get an early start and offer hospitality to adults emerging from estivation in the same field that had been in potatoes during the preceding year. In timothy, however, the volunteer makes slow growth and is infested only late in the season. On the large plants in the grain fields the insect can pass its entire seasonal cycle without resort to the cultivated plants. The volunteers, therefore, constitute a local reservoir from which adult beetles emerging from pupation or estivation disperse to adjacent wild plants or cultivated fields. The writer is of the opinion that the insect may come from such a source



FIG. 1.—Left: "Volunteer" potato plants in buckwheat and rye. Right: Irish Cobbler field

as this when it suddenly appears in well-sprayed fields, as it often does. Occasionally the beetles migrate from defoliated volunteers in great numbers. In one case noted on August 13, 1921, a field of seedling potato plants became so heavily infested with adults from nearby volunteers that it was necessary to hand pick the field in order to save the small plants. One plant harbored 151 adults. The source of this infestation was an adjacent oat field in which the volunteers were very numerous and well developed.

In another example, rye and buckwheat were sown following potatoes, and Irish Cobbler were planted in sod immediately adjoining. The volunteers showed a 78 per cent infestation by the first brood as compared to 11 per cent in the cultivated field. Obviously the emerging adults were satisfied with the volunteers alongside of which they emerged from winter estivation as compared with the

unsprayed cultivated field of the same variety. The subsequent history was a severe infestation of the Cobbler field by the adults of the first brood, although the writer found that many of the adults had remained on the volunteers. Figure 1 is a photograph of these two fields showing their adjacent relation.

The foregoing refers specifically to conditions prevailing in northern Maine.

In western New York, sufficient data are lacking to establish definite relations between the presence of volunteers and infestation. The writer has found volunteer potato plants in wheat fields and their presence there has been noted by growers, but in the season of 1924 they were so few in number, their size so small, and their infestation so light, that no importance could be attributed to them. Whether the season of 1924 was normal in this respect was not determined.

In regard to control, the writer has noted that spraying oat fields with iron sulphate has not materially reduced the number of volunteers, and he does not at this time attempt to suggest a means for their eradication. Consistent crop protection by means of arsenicals and farm practice heretofore considered is, of course, indicated.

SUMMARY

Although great progress has been made in the development of artificial methods for the control of the Colorado potato beetle (*Leptinotarsa decemlineata*), the insect is a persistent and expensive annual pest. Little attention has been centered on methods of protecting the potato crop other than the use of the arsenical stomach poisons.

Certain variable factors operate in a northern latitude to cause a variation from farm to farm, or from field to field, in the percentage of infestation of the beetle.

A study of infested potato fields in western New York and northern Maine indicates these variable factors to be the height of the cultivation ridge, the nature of the previous crop, the earliness in the appearance of the shoot above ground, the deterrent effect of the early sprays, and fluctuations in the numbers of volunteer potato plants.

Farm practice with these factors in mind is recommended.

THE AMMONIA CONTENT OF SOIL, AND ITS RELATION TO TOTAL NITROGEN, NITRATES, AND SOIL REACTION ¹

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INTRODUCTION

Although the ammonia content of soil has been studied by several investigators, in most cases only a few soils have been examined. Russell (12)² found that the ammonia content of unmanured soil varied from 0.5 to 1.6 parts per million; that in manured soils it varied from 4 to 7 parts per million; and that three other field soils contained from a trace to 2.2 parts per million. Matthews (10) reported the ammonia content of nine soils in his work on methods. The maximum amount of ammonia found was 8 parts per million, and the minimum 1.4 parts per million. Potter and Snyder (11) found from 7 to 25 parts of ammonia per million in seven soils. Ellis and Morison (2) found more ammonia in acid peats than in neutral peats, varying from 79 parts per million in the neutral peats to 296 parts per million in the acid peats. This difference was attributed to unfavorable conditions for nitrification. About 50 to 80 per cent of the ammonia in the peats could be extracted with distilled water. Jodidi (6), (7), (8), in his investigation of the chemical nature of the organic nitrogen in the soil, reported on the ammonia content of a few peats and on a plot of soil receiving different kinds and amounts of organic matter. The amount of ammonia varied from 50 to 440 parts per million in case of peats, and from 1.68 to 15.08 parts per million in one series of soil experiments, and from 27 to 33 parts per million in another. Kelley (9) found that the ammonia content of uncultivated soils varied from 2 to 42 parts per million, and that in cultivated soils the variation was from 4.2 to 33.6 parts per million.

EXPERIMENTAL DATA

This investigation was planned to determine whether there is any relation between the ammonia content of soil, soil reaction, total nitrogen, or the amount of nitrates present. In the first experiment 24 soils were used. Five samples were obtained from Professor Truog, of Wisconsin; five from Professor Duley, of Missouri; and the others from various parts of Iowa. All samples were placed in pyrex beakers and left in a covered box in the greenhouse from March 20 until July 5. They were made up to an optimum moisture content occasionally. At three different times during the incubation period they were allowed to become quite dry, with the object of approximating the conditions in fallow plots. All of the soils were moist when the ammonia and nitrate contents were determined. Samples were dried and ground for the total nitrogen determinations.

¹ Received for publication Nov. 3, 1924; issued October, 1925.

² Reference is made by number (*italic*) to "Literature cited," p. 553.

Total nitrogen was determined by the Kjeldahl-Gunning method.

Nitrates were determined by the phenoldisulphonic acid method, using the modification as recommended by the author (4) in case of colored extracts.

Soil reaction was determined by the Truog test (13).

Ammonia was determined by the aeration method as recommended by Potter and Snyder (11), except that large test tubes 1½ by 14 inches were used instead of Kjeldahl flasks to hold the soil suspension, and a cotton plug was inserted between the soil suspension and the absorption bottle to stop the spray formed during aeration. Fifty grams of soil and 100 c. c. of ammonia-free water containing 5 c. c. of a 40 per cent K₂CO₃ solution were aerated for 8 hours at a rate of 200 liters of air per hour. A little paraffin oil was used to prevent foaming. The amount of ammonia was measured by nesslerization, except in case of the larger amounts which were determined by titration.

The results of the first experiment are given in Table I. There is evidently no correlation between the total nitrogen content of the soil and the ammonia content as Russell (12) suggested. Nor, evidently, is there any correlation between the ammonia content and the reaction of these soils. In practically every instance where a large amount of ammonia was present in the soil, the nitrate content was also quite large; however, in some instances the nitrate content was quite large and the ammonia content quite small.

TABLE I.—Ammonia, nitrate, total nitrogen content, and acidity of 24 soils varying widely in physical and chemical properties

No.	Soil type	Source	Acidity	Total nitrogen per 2,000,000 pounds of soil	Nitrogen as nitrate	Ammonia
				Pounds	P. p. m. ^a	P. p. m.
1	Raw peat.....	Manistique, Mich.....	Very strong---	43,800	328.5	32.8
2	Decomposed peat.....	Madison, Wis.....	Not acid.....	24,360	236.5	23.2
3	Clyde silty clay.....	Racine County, Wis.....	do.....	9,260	137.6	7.5
4	Webster clay loam.....	Ames, Iowa.....	do.....	8,630	51.2	6.7
5	Waukesha silt loam.....	La Crosse, Wis.....	Strong.....	6,550	68.2	3.4
6	Shelby loam.....	Jamison, Iowa.....	Slight +.....	5,210	45.5	7.8
7	Tama silt loam.....	State Center, Iowa.....	Strong.....	4,280	125.3	8.2
8	Carrington silt loam.....	Springville, Iowa.....	do.....	3,900	51.2	3.9
9	Shelby loam.....	Udell, Iowa.....	do.....	3,380	196.0	60.7
10	Marshall silt loam.....	Missouri Valley, Iowa.....	Medium.....	3,280	68.0	29.9
11	Carrington loam.....	Calamus, Iowa.....	Strong.....	3,280	41.6	3.7
12	Baxter gravelly loam.....	Polk County, Iowa.....	Slight.....	2,800	128.0	18.7
13	Miami silt loam.....	Rochester, Wis.....	do.....	2,740	44.1	3.5
14	Carrington loam.....	Independence, Iowa.....	Strong.....	2,460	30.4	2.6
15	Grundy silt loam.....	Mountain Pleasant, Iowa.....	Medium +.....	2,460	74.7	7.9
16	Shelby loam.....	Jamison, Iowa.....	do.....	2,240	90.7	6.0
17	Marion silt loam.....	Unionville, Iowa.....	Very strong---	1,990	31.2	7.2
18	Clarksville gravelly loam.....	Lawrence County, Mo.....	Slight +.....	1,700	64.1	46.6
19	Waverly silt loam.....	Polk County, Mo.....	do.....	1,580	28.8	16.6
20	Buckner coarse sand.....	Muscatine, Iowa.....	Strong.....	1,530	28.1	4.5
21	Marion silt loam.....	Boone County, Mo.....	do.....	1,510	36.1	9.6
22	Knox silt loam.....	Missouri Valley, Iowa.....	Not acid.....	1,370	12.0	9.1
23	Lintonia sand.....	Mississippi County, Mo.....	Slight +.....	700	46.0	11.2
24	Carrington sand.....	Vinton, Iowa.....	Medium +.....	560	21.6	3.9

^a P. p. m.=parts per million.

It may be that the ammonia content of soils is influenced quite largely by soil management. Because the treatment of the soils used in Table I was not known, a second series of soils was collected. Eleven samples were taken from plots receiving no treatment on several of the outlying experimental fields, one sample was taken from an onion field near Rudd, four samples were secured near Logan, and a sample of shale was secured near Fairfield.

The results of these analyses are given in Table II. The ammonia content was not affected by the crop, neither was it correlated with total nitrogen or acidity. Soil No. 2, which was growing a crop of onions, had a very high nitrate content; and the ammonia content also was large. The nitrate content varied with the crop grown. It was low in soils growing small grain and higher in case of soils growing legumes and corn (1).

TABLE II.—*Ammonia, nitrate, total nitrogen content, and acidity of several field soils growing various crops*

Sam- ple No.	Soil type	Source	Crop	Acidity	Total nitro- gen in 6½ inches of soil	Nitro- gen as nitrate	Am- monia
					<i>Pounds</i>	<i>P.p.m.^a</i>	<i>P.p.m.</i>
1	Webster silt loam.....	Ames, Iowa.....	Oats.....	Not acid.....	7,870	1.0	8.2
2	Floyd silt loam.....	Rudd, Iowa.....	Onions.....	Strong.....	6,460	153.6	20.6
3	Tama silt loam.....	Newton, Iowa.....	Corn.....	Medium.....	4,680	16.8	12.2
4	Grundy silt loam.....	Mount Pleasant, Iowa.....	do.....	Strong.....	4,660	28.0	9.3
5	do. ^b	do.....	do.....	Medium+.....	2,290	9.0	7.1
6	do. ^c	do.....	do.....	Medium.....	1,290	8.8	4.0
7	do.....	Cedar, Iowa.....	Wheat.....	Strong.....	3,850	1.6	5.6
8	Marshall silt loam.....	Logan, Iowa.....	Alfalfa.....	Very slight.....	3,430	8.6	6.7
9	Putnam silt loam.....	Farmington, Iowa.....	Oats.....	Medium.....	2,700	8.0	7.9
10	do. ^b	do.....	do.....	Strong.....	3,050	5.6	9.3
11	Clinton silt loam.....	Keosauqua, Iowa.....	do.....	do.....	2,990	1.6	7.1
12	Marshall silt loam.....	Logan, Iowa.....	do.....	Not acid.....	2,900	12.8	6.0
13	do.....	do.....	Sweet clo- ver.....	do.....	2,800	12.8	16.8
14	do.....	do.....	Rye.....	do.....	2,740	4.0	5.3
15	Marion silt loam.....	Lowell, Iowa.....	Corn.....	Medium+.....	2,680	24.4	16.8
16	do.....	West Point, Iowa.....	Oats.....	Medium.....	1,880	2.6	4.3
17	Shale.....	Fairfield, Iowa.....	None.....	Very strong.....	500	None.	5.6

^a P. p. m.=parts per million.

^b Subsurface.

^c Subsoil.

In the third experiment a study was made on the effect of temperature and moisture on the ammonia content of four soils which varied widely in reaction and total nitrogen content. Two series of each soil were made up to 25 per cent, 50 per cent, and 75 per cent saturation with distilled water and incubated for 6 weeks. One series was kept at a temperature of 30° C. and the other series at 15°. The results are given in Table III. They do not agree with those presented by Hutchinson (5), who found that ammonia accumulated more rapidly than nitrates at a temperature of 25° to 30°.

It was found in all cases but one that the ammonia content of these soils was lower at the end of the incubation period than at the beginning, while the nitrate content increased on incubation. In case of the Marion silt loam both the nitrates and the ammonia increased at 15° C., but at 30° the ammonia content decreased when the soils were kept at a moisture content of 50 and 75 per cent satu-

ration. This soil is very acid, having a P_H value of 4.9. It also contains a large amount of easily replaceable iron, aluminum, and manganese. It is possible that in this case the nitrifying organisms are inhibited by the unfavorable conditions and that considerable amounts of ammonia can accumulate in the soil.

TABLE III.—*Effect of temperature and moisture on the ammonia and nitrate content of four soils*

Soil type	Acidity	Total nitrogen in sur- face 6½ inches	Nitrogen as nitrate							
			At begin- ning	At end of incubation						
				25 per cent saturation		50 per cent saturation		75 per cent saturation		
				15° C.	30° C.	15° C.	30° C.	15° C.	30° C.	
Webster clay loam.....	Not acid..	<i>Pounds</i> 8,630	<i>P.p.m.*</i> 13.2	<i>P. p. m.</i> 50.4	<i>P. p. m.</i> 65.2	<i>P. p. m.</i> 55.4	<i>P. p. m.</i> 86.6	<i>P. p. m.</i> 70.8	<i>P. p. m.</i> 83.2	
Knox silt loam.....	do.....	1,370	4.9	10.8	16.6	13.9	20.8	14.7	15.9	
Shelby loam.....	Strong.....	3,380	12.5	45.8	90.1	56.4	103.5	78.3	91.2	
Marion silt loam.....	do.....	1,990	11.2	19.8	38.9	22.1	76.8	28.7	69.2	

Soil type	Acidity	Ammonia content							
		At begin- ning	At end of incubation						
			25 per cent saturation		50 per cent saturation		75 per cent saturation		
			15° C.	30° C.	15° C.	30° C.	15° C.	30° C.	
Webster clay loam.....	Not acid.....	<i>P. p. m.</i> 10.22	<i>P. p. m.</i> 5.1	<i>P. p. m.</i> 3.5	<i>P. p. m.</i> 5.0	<i>P. p. m.</i> 3.7	<i>P. p. m.</i> 3.4	<i>P. p. m.</i> 3.7	
Knox silt loam.....	do.....	14.96	.7	1.4	.9	3.4	.34	1.3	
Shelby loam.....	Strong.....	20.19	5.2	2.7	3.9	2.4	3.4	3.0	
Marion silt loam.....	do.....	67.32	74.8	67.4	74.8	41.2	65.9	41.0	

* P. p. m.=parts per million.

During the past season the large amount of rain and very cool weather have kept the soils fairly cold. A large number of field soils have been examined and the ammonia content of none of them has been greater than 40 parts per million, while most of them contained less than 10 to 15 parts per million.

The accumulation of ammonia in soils evidently depends upon the rate of protein hydrolysis and the rate of nitrification, just as the rate of nitrification depends upon ammonification (3), except when ammonia is added to the soil, and the rate at which the nitrates are used by plants and microorganisms. For this reason a method which would determine the amount of easily hydrolyzable protein might give much more accurate information in regard to the availability of soil nitrogen than nitrification tests which are merely an equilibrium depending upon the rate of nitrate formation and consumption, and the results could be determined very quickly. Further investigation is in progress to determine whether such an analytical procedure can be developed which will correlate with crop growth.

SUMMARY

A study was made of the relation between the ammonia content of soils, total nitrogen, nitrate content, and soil reaction. No correlation could be made when the ammonia content of a large number of soils was compared with the total nitrogen content, the accumulation of nitrates, or the soil reaction.

The ammonia content of a soil is evidently in equilibrium with the products of protein hydrolysis and nitrification, just as the nitrate content is in equilibrium with the ammonia content of the soil and the plants and microorganisms.

The ammonia content of most field soils does not exceed 20 parts per million, and the majority of soils contain less than 10 parts per million. Occasionally the ammonia content of some soils may be as high as 60 or 70 parts per million. This may be due to conditions being unfavorable for nitrification or to vigorous ammonification, or it may be the result of both of these factors operating simultaneously.

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LOCALIZATION OF THE RESPONSE IN PLANTS TO RELATIVE LENGTH OF DAY AND NIGHT¹

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INTRODUCTION

In earlier papers² it has been seen, with many species of plants, that modification of the duration of the daily illumination period seems to have definite formative effects on growth and of bringing about certain well-defined chemical changes within the plant.

As an aid in attempting to determine the manner in which these formative effects are brought about, it is of considerable interest to know the results when different parts of the plant are exposed to different periods of illumination. In other words, when two parts of the same plant are exposed to different illumination periods, will each part show the characteristic response to its particular light period, more or less independently of the action of a different light period on the other plant part? This question has already been answered in part in an earlier paper,³ but in recent experiments the results have been considerably extended.⁴

In the earlier work, specimens of *Cosmos bipinnatus* Cav. were cut back to the first node above the cotyledons, from which two branches were allowed to grow under a daily illumination period of 16 hours. At the end of a month a vertical cardboard screen was arranged between the two branches of each plant, one branch being exposed only to the natural daylight period of winter, while the other branch was exposed to electric illumination from sunset till midnight, in addition to the natural illumination. Each branch showed the characteristic response to its illumination period—that is, the branch exposed to the short illumination period promptly flowered and developed seed, while the branch exposed to the longer light period remained vegetative to the end of the test.

EXPERIMENTAL DATA

The tests previously reported had to do with two coordinate branches of a plant exposed to different day lengths. The present experiments deal with different portions of the primary stem exposed

¹ Received for publication Oct. 16, 1924; issued October, 1925.

² GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

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³ GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

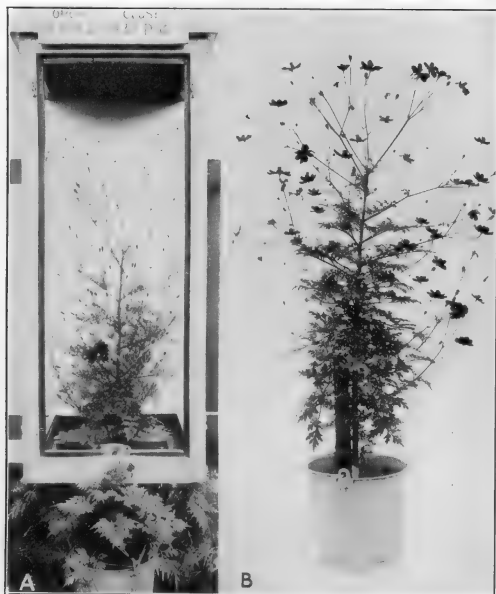
⁴ In this paper and in earlier experiments dealing with plants, different parts of which have been exposed to different illumination periods, only short-day plants, flowering in response to relatively short daylight periods, have been used. Since the present paper was submitted for publication the work has been extended to a series of typical long-day plants, in which flowering is induced by exposure to relatively long daily illumination periods. Results secured to date (September 1, 1925) indicate that these plants, like the short-day plants, show localized responses, flowering only on those stems receiving a long daily illumination period. Publication of the details of these tests is deferred to a later date.

to different light periods. For these tests *Cosmos sulphureus* Cav., a typical short-day plant, has been used. When exposed to a day-light period of 12 hours or less, this species flowers readily, while it tends to remain in the vegetative stage for an indefinite period when exposed to a daylight period in excess of 13 hours (pl. 1 and pl. 2, B). Flowering normally begins at the apex and rather rapidly extends downward along the axis, numerous axillary flowering branches being developed.

The first experiment was made to ascertain the results when the lower portion of the axis is exposed to a short daily light period while the upper portion is exposed to a long one. The test was begun in the greenhouse in winter. Seedlings were grown with a daily illumination period of 16 hours till $3\frac{1}{2}$ months old, electric light being used to prolong the light period after sunset. From the beginning of the experiment on January 7, the control plants were exposed to only the natural daylight, which was of about 10 hours' duration, and they began flowering February 9. Around the upper portion of the axis of the test plant a box with open front and top was so arranged that it could be raised as the plant grew taller. A 100-watt electric light placed within the box and 1 foot above the apex of the plant was turned on daily from sunset till midnight, thus giving about 16 hours of illumination daily. The front of the box about the upper part of the plant was kept covered with black cloth while the electric light was on so as to prevent the light reaching the lower part of the test plant and the controls. The upper portion of the plant inclosed in the box showed the usual response to prolonged daily illumination. It remained in the vegetative state and continued to increase in stature. The lower portion of the stem, exposed only to the short daylight period of winter, soon developed flowering branches at the nodes, the first blossom opening March 5. The appearance of the test plant and a control on March 9 is shown in Plate 2, A. Both the upper and lower portions of the axis have shown the characteristic responses to their respective light periods and the normal position of the flowering portion of the stem has been reversed.

The treatment of the test plant was continued till May, when the days had become sufficiently long so that the use of the electric light could be discontinued. The plant was then transplanted outdoors. Although the entire above-ground part of the plant was exposed to the long days of summer, the lower portion continued to flower throughout the summer, while the upper portion continued its vegetative growth, as shown in Plate 2, B. By the middle of August the plant had reached a height of 11 feet. By October 1, in response to the decrease in length of day, the entire upper portion of the plant passed into the flowering condition, flower buds developing rapidly on all branches.

In this experiment and those subsequently described, as well as in the one mentioned in the opening paragraph, it would seem that any possibility of effects due to differences in water supply, plant nutrients, or other soil factors has been eliminated. It will be observed that in the earlier test the two branches growing from the same node, but differently illuminated, obtained at all times their supply of the soil solution through the same mother stem. In the present case the soil solution reaching the upper portion of the



A.—Yellow Cosmos, upper portion of which was placed in the light-proof chamber on June 13 and received thereafter only 10 hours of light daily. The lower portion of the plant received the light of the full length of day. In response to the short day, flower buds were visible in the upper portion of the plant by June 25, and the first open blossom appeared July 9. The lower portion, exposed to the long day, remained vegetative throughout the summer.

B.—Sister plant of the individual shown in Plate 1, A. This plant was grown under long-day conditions until June 13, when it was given 10 hours of illumination daily. Flower buds could be seen June 28, and the first open blossom appeared July 10. Photographed July 16. This plant represents the typical behavior of yellow Cosmos when exposed to a short day. The behavior of the plant when exposed to long days is illustrated by the upper portion of the individual shown in Plate 2, B.



A.—Plants from seed which germinated September 25. Seedlings were grown in a greenhouse artificially lighted from sunset till midnight each day until January 7 when test was begun. From that date control plant at right was exposed to the natural daylight period of winter, and began flowering February 9. Beginning January 7 lower portion of plant at left received the natural daylight only, while the upper portion received electric illumination from sunset till midnight in addition to daylight. In response to the short-day exposure, the lower portion showed flower buds by February 14, and the first open blossom appeared March 8. Responding to the long-day exposure, the upper portion remained in the vegetative condition and continued to increase in height. Photographed March 9.

B.—Later stage of development of the Cosmos plant shown at left in 2, A. On May 7, when the natural length of day had increased sufficiently to inhibit flowering, the electric illumination was discontinued and the plant was transplanted outdoors. Vegetative development of the upper portion continued, and when photographed August 18 the plant had attained a height of 11 feet. The lower portion of plant, however, continued to flower through the summer, as shown here.

primary stem, which remained in the vegetative stage throughout the exposure to long days, necessarily passed through the lower portion of the stem, which, nevertheless, continued in the flowering condition. Moreover, the same is true of the organic materials passing from the upper vegetative portion of the stem downward to the roots.

The initial experiment having given such interesting results, it seemed desirable to study further the possibilities in localizing the reproductive and vegetative phases of development in different portions of the axis in the individual plant. For this purpose a series of tests was conducted during the summer, when the days are long. To control the light exposures of the different parts of the plants light-proof boxes of suitable size, with three sides removable, were set up. Ventilation was provided for by inserting into the top and bottom or the upper and lower parts of the back of each box or compartment a 3-inch galvanized-iron elbow to which were attached two similar elbows in such way as to form a double bend. These doubly curved pipes, blackened inside and outside, proved to be very satisfactory for the purpose in view. The top or bottom of each box, as required, was provided with a slot leading to an opening in the center into which the primary stem of the test plant could be slipped and the slot then closed. A front view of the cases, showing the plants in position, is seen in Plate 3. The series of compartments here shown make possible the exposure of top and bottom of the plant axis to different day lengths, the exposure of the central portion of the axis to a day length differing from that received by the upper and lower portions, and, finally, exposure of the upper part of the axis to continuous darkness day and night while the lower portion is exposed to a regulated day length.

Seedlings of *Cosmos sulphureus* were grown during the spring months in a greenhouse in which electric light was used from sunset till midnight. Early in June the plants were set in 10-quart metal buckets and placed outdoors in preparation for the tests. As a control plant, to show the normal response to a short day, an individual was placed under a 10-hour day, beginning June 13, and the first blossom opened July 10. The appearance of the plant on July 16 is seen in Plate 1, *b*. None of the plants left in the open showed any indications of flowering till October. The upper portion of the test plant shown in Plate 2, *A*, may be taken as illustrating the typical behavior when exposed to a long day.

Beginning June 13, the upper portion of one of the plants was exposed to a 10-hour day while the lower portion was exposed to the full period of daylight. For this experiment the light-proof box shown in Plate 3, *e*, was used. This treatment was continued till September 23 when the plant was transferred to the greenhouse. On the upper portion of the plant exposed to the short day, flower buds could be seen June 25 and the first blossom opened July 9. No flowers or flower buds appeared on the lower portion of the stem, exposed to long days, except at the node immediately below the bottom of the box. (Pl. 1, *A*.) At this node, which was about 1 inch below the base of the dark box, a single blossom opened August 11. Flower buds finally appeared in the lower portion of the stem October 19, after the plant had been transferred to the greenhouse.



Series of ventilated, light-proof chambers used in regulating the duration of the daily period of illumination received by different portions of the plant. Chamber *c* is used in exposing the upper portion of plant to a short day while the lower portion is exposed to the full length of day; *d* is employed in exposing the lower portion of the plant to a short day while the upper portion receives the light for the full length of day; the two chambers, one on top of the other, *e*, *f*, are used to maintain the upper portion of the plant in continuous darkness, while the lower portion is subjected to either a long or short day, as desired; the pair of chambers *g* are arranged for exposing both the upper and lower portions of plant to a short day, while the central portion receives the light of the full day length; the chamber *a* is arranged for reversing the last-named light exposures

Beginning on the same date, another test was made in which the treatments just described were reversed, the upper portion of the plant being exposed to the full period of daylight, while the lower portion was exposed for only 10 hours daily (pl. 3 *a, d*). Flower buds appeared in lower portion of plant July 3, and the first blossom opened July 17. No flower buds appeared on upper part of plant until October 1 after it had been transferred to the greenhouse. Here, again, the vegetative and the flowering zones of the axis were sharply delimited by the two light treatments.

The last experiment, in conjunction with that first described, shows that flowering has been readily induced on the lower portion of the stem by exposure to a short daily light period, while flowering in the upper part of the stem was inhibited by exposure to a long daily period of illumination, whether the light was wholly natural or partly artificial and of low intensity.

Having shown that flowering was readily confined to either the upper or the lower portions of the stem by regulating the duration of the respective light periods, it was decided to study the somewhat more complex situation in which the central portion of the stem is exposed to a light period differing from that to which the upper and lower portions are subjected. Beginning July 11, the upper and lower portions of the stem of a *Cosmos* plant were allowed to receive only 10 hours of light daily while the central portion of the stem was exposed to the full period daylight. This was accomplished by using two light-proof cases, one arranged 12 inches above the other (pl. 3, *b*). Flower buds were visible on the lower portion of the plant July 30 and on the upper portion July 26, while the first open blossom on the lower part appeared on August 21 and on the top part on August 11. No flower buds appeared in the central zone of the stem until the plant had been transferred to the greenhouse, except that at the node immediately below the base of the upper light-proof case a few buds appeared in late August. The flower buds at this node, which was scarcely an inch below the base of the dark compartment, were unable to develop and soon perished. Each portion of the stem responded to its particular light exposure in characteristic manner, without material interference from adjoining sections responding in turn to a different light period (pl. 4, *A*). This experiment furnishes additional evidence that the action of a short light period in inducing flowering does not extend upward to parts of the plant simultaneously exposed to a long light period; and, similarly, this action in inducing flowering extends downward in the stem only a very short distance at most when the lower portion is exposed to a long light period.

In another test begun July 13, the treatments just described were reversed, the middle portion of the stem being subjected to a 10-hour day and the upper and lower portions being exposed to the full length of daylight (pl. 3, *a*). Flower buds were visible on September 10, on the central portion exposed to a 10-hour day, and the first blossom opened October 18. No buds appeared on the upper and lower portions exposed to the full length of day till after the plant had been transferred to the greenhouse. Because of the low height of the light-proof chamber the illumination within when the sides were open was poor and undoubtedly inadequate, and it was probably for this reason that unfolding of the flower buds was

materially delayed. In any event, the localized effect of the long and short daily illumination period is plainly evident.

Extensive data presented in the earlier papers, already referred to, make it clear that, when compared with the action of a longer daily illumination period, the effects of a shorter light period may fail to show any tendency to approach those produced by total darkness. It seems quite clear that, in general, the formative action of light on the aerial portions of the plant may readily extend to parts which are under ground and therefore excluded from light, influencing their development in various ways. That distinctive differences in the formative effects of long and short daily illumination periods may be thus transferred to subterranean organs in the case of the potato has been shown in a previous paper.⁵ On the other hand, the above tests indicate that the local action of a relatively short daily illumination period in initiating flowering in yellow Cosmos is not extended to an adjoining portion of the plant which is exposed to a light period unfavorable to sexual reproduction. It seemed worth while, therefore, to determine the comparative effects of a short and a long daylight period when acting on the lower portion of the plant, in influencing the activities of the upper part exposed to continuous darkness. The first tests were carried out during the summer of 1923.

Using a combination of two light-proof chambers, one arranged immediately above the other as shown in Plate 3 c, the upper portion of a Cosmos plant was maintained in continuous darkness, while the lower portion was exposed to 10 hours of daylight. These treatments were begun on July 6, and on July 26 the upper portion was restored to the natural length of daylight by opening the dark chamber. During the period of darkening, the portion of the stem excluded from light increased in length from 3 inches to 21 inches. When returned to the light this part of the stem was badly etiolated, had lost the power to stand erect, and no flower buds could be seen (pl. 4, B). During the time the upper portion was darkened, the lower portion of the stem which was exposed to the 10 hours of daylight wilted badly, but this condition soon disappeared after the upper portion was restored to the light. Flower buds had appeared on the lower portion by July 18, and the first blossom opened August 11. On the upper part of the stem, which had been darkened, flower buds appeared by August 20, and the first blossom opened September 7.

It will be observed that the upper portion of the plant, restored to the full natural length of daylight after the exposure to continuous darkness for 20 days, flowered as soon as it had recovered sufficiently to do so. It appears, therefore, that the action of the short day on the lower portion of the stem was in some way transmitted to the upper portion which was darkened. These results are in line with the fact just mentioned that the formative action of light may be transmitted to parts of the plant which receive no light. This is in contrast, however, with the relations which obtain when one part of the plant is exposed to a short light period and the other to a long period. The experiment was repeated, beginning August 20 and maintaining the upper portion of the plant in continuous darkness for 28 days. The results were similar to those of the first test.

⁵ GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. *Jour. Agr. Research* 23: 891. 1923.



A.—Yellow Cosmos plant, the upper and lower portions of which were placed in light-proof chambers on July 11 and received thereafter 10 hours of light daily, while the central portion continued to receive the light of the entire summer day. Both top and bottom of the plant responded in characteristic manner to the short day and soon flowered, as shown in the photograph. The central portion, on the other hand, remained vegetative in response to the long day. Photographed September 11

B.—Yellow Cosmos plant, the upper portion of which was maintained in total darkness from July 6 to July 26 and then restored to the full length of day. While the upper portion remained in continuous darkness the lower portion was exposed to a 10-hour day. Flower buds were visible in the lower portion July 18, and the first blossom opened August 11. The upper portion was badly etiolated by the darkening, but soon recovered when restored to light and was showing flower buds by August 20 and an open blossom on September 7 in response to the supposed transmitted effect of exposure of the lower part of the plant to a short day

In the summer of 1924 further tests were conducted with yellow Cosmos. The seed germinated January 26. The preliminary treatment of the seedlings was the same as in the tests of the preceding year. The control plants remaining outdoors through the summer showed flower buds October 1. Tests involving three different light treatments were made: (1) upper portion of plant subjected to continuous darkness, and lower portion exposed to 10 hours of daylight daily; (2) upper portion exposed to continuous darkness, and lower portion allowed to receive all the daylight of summer days; (3) entire plant maintained in continuous darkness, and subsequently returned to all the natural illumination of summer days.

The first series, which was carried out to confirm the results of the experiments made the preceding year, was begun June 3. The upper portion of the plant was restored to the full length of daylight on July 9. Possibly because of the somewhat cooler weather which prevailed during this time, it was possible to prolong the exposure to darkness beyond the lengths of the exposures in the previous experiments, without irreparably damaging the darkened part of the plant. The lower part of the plant, exposed to 10 hours of daylight, showed flower buds June 28, and the first blossom opened July 15. In this instance, the upper, darkened portion likewise showed flower buds on June 28, and the first open blossom on July 15. The test was repeated, beginning July 9, and in this test it was possible to continue the exposure to total darkness for only 19 days. The lower part of the plant, in response to the 10 hours of daylight, showed flower buds July 22, and first open blossom August 8; while the upper, darkened part showed flower buds August 26, and first open blossom September 27.

The first test of the second series was begun May 31. The upper portion of the plant was restored to the full length of daylight on July 3, after having been darkened for 34 days. The lower portion of the plant, exposed to full daylight, showed no flower buds through the summer, and the same was true of the upper part which had been darkened. Flower buds finally appeared in the top of the plant at the same date as in the controls, namely, October 1. In a second test, begun July 3, the top of the plant was excluded from light for a period of 25 days while the lower portion, as previously, received throughout the test the light of the full length of day. No flower buds appeared on the plant till October 1.

In the third series, three plants were kept in darkness from July 12 to July 22. When returned to outdoor conditions, the plants were in a critical condition, due to advanced etiolation, and two of them perished. The third individual survived, turned green, and resumed vegetative development. No flower buds appeared till October 1.

DISCUSSION OF RESULTS

These tests furnish no evidence that darkness in itself is capable of initiating flowering. Considering the whole plant, under the conditions of the test, exposure of Cosmos to continuous darkness failed to result in reproductive activity, while it has been seen that exposure to a short period of daylight seemed to promptly promote flowering and fruiting, and that exposure to a long period of daylight apparently inhibits these processes. Likewise, in the tests in localiz-

ing the action of the light period, exposure of the upper portion of the axis to continuous darkness apparently did not in itself favor formation of flower buds, for these appeared only when the lower portion of the axis was exposed to daylight for 10-hour periods, and failed to form when the lower portion was exposed to a long day. On the other hand, mere absence of light seemingly does not inhibit the laying down of flower buds in response to the transmitted influence of a short daily light period acting on an adjoining portion of the axis. A short daily illumination period may exert the same local effect in initiating sexual reproduction, without material interference from the action of total darkness or of a long daily illumination period on other parts of the plant, as when acting upon the green portion of the plant as a whole. The same is true of the local action of a long daily light period in maintaining vegetative development and inhibiting flowering and fruiting.

SUMMARY

In earlier investigations it was seen that when two coordinate branches of an individual plant of *Cosmos bipinnatus* are exposed to light for daily periods of different length each branch responds in characteristic manner to its particular light period more or less independently of the other branch. Continuing the investigations, experiments have been made with *Cosmos sulphureus*, a typical short-day plant, in which different portions of the primary stem were exposed to different daily periods of illumination, and, in some instances, to continuous darkness.

When the upper portion of the plant was exposed to the full length of day of summer while the lower portion received only 10 hours of light daily, the latter promptly flowered while the former remained vegetative. These localized responses might, it is supposed, continue for several months under favorable conditions.

Thus each portion of the axis has responded to its particular light period, in much the same manner as if it had been a separate plant.

Definite localization of the response was likewise obtained when the upper portion was subjected to a short daily period of illumination, and when the lower was exposed to a long one, and the former flowered readily while the latter continued to develop vegetatively.

When the central portion of the axis was exposed to a long day while both the lower and uppermost portions were subjected to the action of a short day, flowering was initiated in both the latter portions of the axis while the central portion continued to develop only vegetative shoots, further illustrating the possibility of sharply localizing the response to length of day.

On the other hand, when the central portion of the axis was exposed to a short day and the upper and lower portions to a long day, flower buds appeared in the former while both of the latter remained vegetative.

When the upper portion of the stem was excluded from light continuously for a period of 3 to 5 weeks while the lower portion received the light of a short day and consequently was forced into flowering, formation of flower buds was induced in the upper, darkened portion, and open blossoms appeared after the top had been returned to the

full length of day. When, however, the lower portion of the axis received the light of the long summer day and therefore was prevented from flowering, while the upper portion was maintained in continuous darkness, the latter formed no flower buds and resumed vegetative activity when returned to the light.

When the entire plant was excluded from the light for a period of 10 days and then returned to the action of the long day, flower buds did not appear.

These tests seem to indicate that, under the conditions, continuous darkness in itself does not definitely initiate flowering, but that, on the other hand, it does not necessarily inhibit formation of flower buds in response to the action of a short daily light period on another part of the plant.

BEHAVIOR OF PHYTOPHAGA DESTRUCTOR SAY UNDER CONDITIONS IMPOSED BY EMERGENCE CAGES¹

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INTRODUCTION

The Hessian fly, *Phytophaga destructor* Say, has long been recognized in America as a wheat pest of primary importance, and even before its scientific description by Thomas Say in 1817 it had attracted considerable attention and was referred to under the popular name of Hessian fly. Naturally such an insect pest has been the subject of much published discussion as well as almost every conceivable type of investigation. Many excellent accounts of the life history, food habits, natural enemies, control, and other interesting phases in the life of this insect are readily available and therefore need not be repeated. Osborn (?)² has given a very comprehensive treatise on the Hessian fly in America, with a complete bibliography of all important papers up to 1898. For contributions appearing since that time full references will be found in the Bibliography of American Economic Entomology (1, 8).

The determination of the time and extent of the emergence of adult flies from flaxseeds under varying conditions has always been an important factor in the study of this phase of the life history of the Hessian fly, and particularly in reference to various schemes for control. The present discussion deals with the design of a number of emergence cages, and the determination of the relative efficiency both as to approach to natural conditions and ability to recover emerged flies.

EMERGENCE CAGES

Although many types of cages have been suggested from various sources, the selection of type, actual design of cages, and accomplishment of results may be considered as original. The six types of emergence cages selected were as follows:

TYPE A.—Cone-shaped, frame of sheet tin covered with 18-mesh pearl screen wire. Base circular, of diameter sufficient (40.6 inches) to make it inclose 1 square yard. Altitude 30 inches, and top terminating in a sheet-tin cone with 0.5-inch hole at the small end. Over this hole is fitted a small screen trap for catching flies that emerge from the cage through the hole in the top (fig. 1, A).

TYPE B.—Square, base inclosing 1 square yard. Height, 18 inches. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Top is painted with thin tanglefoot and made to open upward to facilitate counting of flies caught on the tanglefoot (fig. 1, B).

¹ Received for publication Oct. 7, 1924; issued October, 1925.

² Reference is made by number (italic) to "Literature cited," p. 574.

Type C.—Square, base inclosing 1 square yard. Height, 18 inches. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Top is made to open upward to facilitate counting of flies. The entire cage is lined with cheesecloth. Four single sheets of tanglefoot fly paper are fastened to the lower side of the top (fig. 1, C).

TYPE D.—This is a light-proof box of 0.5-inch pine, with base inclosing 1 square yard. In later experiments this type was made of a wood frame covered with tar paper. Two 29-millimeter glass vials were inserted through holes bored in the south side of the box (fig. 1, D).

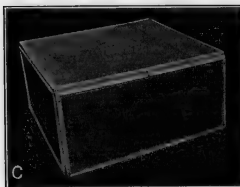
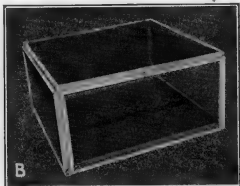
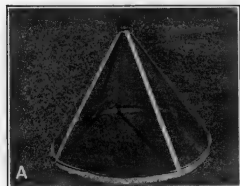


FIG. 1.—A, emergence cage for the Hessian fly, type A, having cone-shaped frame of sheet tin, covered with screen wire; B, emergence cage, type B, having square frame of cypress, covered with screen wire; C, emergence cage, type C, having square frame of cypress, covered with screen wire and lined with cheesecloth; D, dark emergence cage having frame of wood, made light-proof with tar-paper covering.

TYPE E.—May be described as A-shaped, with square base inclosing 1 square yard. The two sloping sides are 1 yard square each and the ends A-shaped. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire. Door, made by hinging one of the sloping sides at the top, is painted with thin tanglefoot, and the cage set so that this door faces south (fig. 2, A).

TYPE F.—This may be described as A-shaped with base square, inclosing 1 square yard; the two sloping sides 1 yard square each, and the ends A-shaped. Frame of 1 by 2 inch cypress, covered with 18-mesh pearl screen wire, with the exception of the door, which is made by having one of the sloping sides hinged at the top. This door is covered with 12-mesh pearl screen wire and painted with thin tanglefoot. The cage is set so that this door faces south (fig. 2, D).

FIRST TRIAL, NASHVILLE, ILL., APRIL 10 TO 30, 1917

The cages just described were arranged in a row extending east and west. Each covered 1 square yard of heavily infested winter wheat. There was reason to believe that each square yard was as nearly uniformly infested as was possible to obtain. However, there is some question as to whether an approximately equal number of flies might be expected to emerge in each cage. The cages were set in position April 9. During the 21 days of the experiment the total number of flies taken from each cage was as follows: A, 65; B, 63; C, 8; D, 134; E, 54; F, 61; total for all six cages, 385.

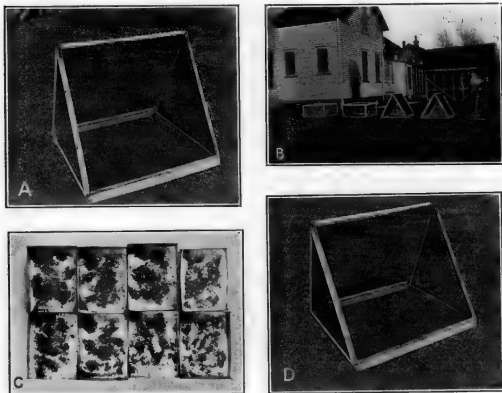


FIG. 2.—A, emergence cage for the Hessian fly, type E, having A-shaped frame of cypress, covered with 18-mesh screen wire; B, emergence cages in place for outdoor experiment; C, 12,000 "flaxseeds" or puparia used in indoor experiment; D, emergence cage, type F, having A-shaped frame of cypress covered with 18-mesh screen wire, except door, which is 12-mesh

The results obtained indicate that cage D was approximately twice as efficient in recovering flies as the best of the other cages. Cage C shows up very poorly, whereas the other cages are so close together in number of flies recovered that other considerations might be allowed to influence the selection of one of these cages for any particular purpose. A comparison of temperature, evaporation, and rainfall in each cage with that of natural conditions outside, or normal, was made. Temperature variations were determined by radiation thermometers placed close to the soil surface, and are shown in degrees plus or minus from the normal. Variations in evaporation were determined by compensating atmometers, and are shown in cubic centimeters plus or minus from the normal; the evaporation for each preceding 24 hours being recorded at 8 a. m. Variations in rainfall were determined by espe-

cially adapted standardized rain gauges and are shown in inches plus or minus from the normal, the amount for each preceding 24 hours being recorded at 5 p. m. Readings for each factor were made, and the accumulated variations are given in Table I.

TABLE I.—Accumulated variation 19-day period, April 12 to 30, 1917

Factor	Cage A	Cage B	Cage C	Cage D	Cage E	Cage F	Time
Minimum temperature (°F.)---	+34.5	+32.0	+22.5	+51.5	+26.0	+31.5	
Temperature (° F.)-----	+9.5	-5.0	-26.0	-25.0	-2.0	-2.5	8 a. m.
	-21.5	-33.0	-103.0	-141.5	-39.5	-17.5	12 m.
	-8.0	-15.0	-51.0	-65.0	-26.0	-24.0	4 p. m.
Evaporation (c. c.)-----	-80.4	-110.4	-223.2	-282.1	-118.8	-104.9	8 a. m.
Rainfall (inches)-----	-.49	-.16	-1.57	-3.24	-.53	-.59	5 p. m.

SECOND TRIAL, LA FAYETTE, IND., MARCH 15 TO 24, 1920

In order to make an extra trial, it was decided to run the cages indoors, thus forcing the adult flies to emerge by artificial heat. The cages were arranged in the laboratory under conditions as nearly uniform as possible.

The disadvantage pointed out in the first trial, namely, the existence of an uncertainty as to whether about the same number of flies emerged in each cage, was recognized, and an attempt made to eliminate it. Twelve thousand "flaxseeds" (fig. 2, C) were taken from wheat plants killed by the regular fall brood of the fly, and after being thoroughly mixed so that the entire lot would be uniform, they were divided into smaller lots, and 1,500 each were placed over moist soil in small rectangular tin boxes, and then exposed, one box in each cage. An additional 1,500 flaxseeds were placed in tin parasite boxes, and the adult flies allowed to emerge into glass vials. By counting these daily, and also those in the boxes, a suitable control was obtained on the total number which could reasonably be expected to emerge from each 1,500 flaxseeds in each of the cages.

Flies were counted and removed from the cages daily, as in the preceding trial, except that the record of males and females was kept separate, as follows:

Cage A, 205 females, 539 males, total 744; B, 127 females, 453 males, total 580; C, 21 females, 32 males, total 53; D, 307 females, 355 males, total 662; E, 195 females, 527 males, total 722; F, 136 females, 435 males, total 571; control, 512 females, 601 males, total 1,113.

No attempt is made to consider this trial as being conducted under any other than extremely unnatural conditions; however, it does give considerable information on the subject. It brings out the fact that although moisture and temperature may be the controlling factors in the emergence of adult flies, wind is certainly of some importance in the determination of cage efficiency. Indeed, the percentage of recovery is very remarkable in most cases, the exception being cage C, which will undoubtedly prove to be too inefficient to merit further consideration. Another remarkable point brought out in this experiment is the fact that from 1,500 flaxseeds used in the control, 1,113 adult flies were obtained, thus indicating that the very high rate of 74 per cent of the flies survived the winter, parasitism, and other

fatalities. Of the total number of flies emerging in the control, 601, or 54 per cent, were males, and 512, or 46 per cent, were females, in this case indicating a very fair degree of equality in the proportion of sexes. Another interesting point, although to be expected, is that of the recovery of the greater percentage of males in comparison with the females in each cage except the dark cage D. Excepting the cage C, there does not seem to be any choice indicated by the percentage of efficiency when the cages are run indoors.

A hygrothermograph was run in cage A, and temperature and humidity charts (Table II) were kept.

TABLE II.—*Temperature and humidity, La Fayette, Ind., March 15 to 24, 1920*

Date	Temperature			Humidity		
	Minimum	Maximum	Average ¹	Minimum	Maximum	Average ¹
	° F.	° F.	° F.	Per cent	Per cent	Per cent
Mar. 15	65	75	70.5	38	49	43.8
Mar. 16	60	72	65.8	35	40	38.4
Mar. 17	58	70	64.4	34	36	34.7
Mar. 18	58	68	64.2	36	39	37.5
Mar. 19	62	68	64.6	37	44	39.7
Mar. 20	58	74	68.1	35	38	36.2
Mar. 21	62	77	71.4	33	36	34.2
Mar. 22	65	77	71.1	33	37	35.1
Mar. 23	68	76	73.5	31	37	34.2
Mar. 24	69	78	72.4	36	44	40.6

¹ The average is the arithmetical mean of readings at 2-hour intervals.

THIRD TRIAL, LA FAYETTE, IND., APRIL 22 TO MAY 12, 1920

The third trial was made in the same manner as the second, with the two main exceptions that the cages were placed under natural conditions outdoors and arranged in a row, as shown in Figure 2, B. Flaxseeds for this trial were collected and kept under as nearly natural conditions as possible until placed in the cages. One thousand flaxseeds were placed in each cage and protected from the sun's rays by small canopies of cheesecloth over each box. The results obtained are as follows: Cage A, 78 females, 191 males, total 269; B, 74 females, 88 males, total 162; C, 20 females, 12 males, total 32; D, 130 females, 233 males, total 363; E, 49 females, 100 males, total 149; F, 39 females, 55 males, total 94; control, 524 females, 240 males, total 764.

Cage D is again indicated to be the most efficient, and it is unfortunate indeed that this cage should impose conditions which are further from natural conditions than those of any of the variously devised cages of the experiments. Although in this trial, as proved by the control, the emergence was heavy, the number of flies recovered by the cages falls far below the indoor records. From 1,000 flaxseeds of the control 764 flies were obtained, a yield of 76 per cent. This comes very close to the indoor record, which was 74 per cent.

Attempts at further trials of these cages on May 11 and again on June 18, 1920, were made by placing flaxseeds in refrigeration to retard their development until after the natural emergence had occurred in the field. For some reason a sufficient emergence was

not obtained from these two lots of flaxseeds, and the scattering results will not be included.

Again, on September 15, 1920, another lot of flaxseeds was started in the cages, but owing to the freakish circumstance that adults emerged about 20 days later in the season than usual the number finally recovered by the cages was too small to give any data worth recording. In a faithful daily examination of the cages made throughout the period, it was discovered that ants were attacking and carrying away the flaxseeds; because of the unforeseen and extremely unusual delay in emergence, they were able to destroy quite a large number of them. This is a condition likely to occur whenever the cages are used, and it can not be considered other than a disadvantage.

DISCUSSION

Of a total of 38,500 flaxseeds used in these experiments, records were made on 17,500, from which one may expect 13,125 flies to have emerged, and of these 6,278 were recovered by the various cages. An additional 385 were recovered by the cages in the first trial, making a total of 6,663 flies recovered and counted during the experiment.

Probably the best arrangement of the information obtained in the preceding experiments, and one by which a comparison can be made for selection of a cage for any special purpose, is that of a tabulation of the relative efficiency of the cages used. Table III shows, on a percentage basis, the conditions in each cage as compared to natural conditions—the natural temperature, evaporation, and rainfall being taken as 100 per cent. Efficiency in recovering flies is shown by the average per cent recovered from the number reasonably supposed to have emerged in each cage. The results from the indoor trial were not included in this rating, since conditions under which this particular experiment was run were unique.

TABLE III.—*Cage efficiency, on percentage basis*

Cage	Temperature				Evapo- ration	Rain	Flies recov- ered
	Mini- mum	8 a. m.	12 m.	4 p. m.	8 a. m.	5 p. m.	
A-----	95	99	99	99	76	85	35
B-----	96	99	97	90	66	95	21
C-----	97	97	92	96	33	58	4
D-----	94	97	89	95	15	00	48
E-----	97	99	97	98	64	84	20
F-----	96	99	99	98	68	82	12
Control-----	100	100	100	100	100	100	100

It is found that cage A comes the nearest to being suitable for the determination of the date when flies are emerging in the field. For the actual recovery of flies, the dark cage D was the most efficient. For counting the flies, cages B, E, and F have their advantages, whereas cage C seems to be entirely impractical, as is shown in Table III.

PRACTICAL APPLICATION

A discussion of the use that has already been made of this information on emergence cages in investigations that have been conducted subsequent to those of cage development may be appropriate. The dark cage D has been the one adopted in most cases, and many unpublished data on various treatments of stubble as a fly control have been compiled. Although this may be said to be the primary purpose of these cage experiments, it was also desirable to have a cage for use at "emergence stations" in comparison with migration screens and egg counts, as has been described by Gossard and Eastwood (4), Gossard and Parks (5), and later at "observation stations" as described by Drake, Fenton, and Butcher (3). The dark cage has given very satisfactory results in both cases, and when properly manipulated under the immediate direction of a trained entomologist, serves as a good indicator of Hessian-fly emergence.

It might be well to discuss briefly the results of experiments with emergence stations and the part played by emergence cages, the detailed report of which will be published later. Such stations have been in operation at LaFayette, Ind., and Centralia, Ill., during the fall emergence of the fly for the years 1919 to 1923 inclusive, that at the latter location being under the immediate observation of W. B. Cartwright, of the Bureau of Entomology, United States Department of Agriculture.

After this rather extensive use of emergence cages, migration screens, egg counts, and flaxseed examinations, dependence is now placed almost entirely on egg counts and flaxseed examination to give the emergence records which are most closely associated with infestation of wheat. The reasons for this are the advantages of simplicity, high degree of responsiveness, economy, and accuracy. Many midges closely resemble Hessian flies in cages and on migration screens, whereas there is very little chance of mistaking anything for a Hessian fly egg. The flaxseed examination to determine the status of pupation proved to be a very important contribution to the emergence-station activities.

The results obtained by this series of emergence stations have certainly demonstrated their value in the scientific study of this phase of the life history of the fly. Confidence in the ability thus to predict the safe time to sow wheat any one year has not been experienced to nearly the extent that has been the case elsewhere. Without flaxseed examination, a peak of emergence may be obtained, but there is nothing to indicate that there may not be another peak. Even with the status of pupation determined by flaxseed examination, actual emergence must occur before it can be heralded as such. By the time complete pupation and emergence have been determined, the sowing date will have been delayed five to seven days longer than necessary, a very vital matter to the farmer with some sizable acreage to sow. If regular sowing time arrives and passes and still a large proportion of the fly remains in the larval stage in the flaxseeds, just what policy is to be adopted? There can be no assurance that such larvae will remain in that stage until the following spring. They may pupate the very next few days after the safe sowing date has been announced, and thus emerge at exactly the right time to do the most damage to young wheat. Such cases have occurred too

frequently (2, 6), and during the last five years in the East Central States have constituted the only really serious factor affecting the reliability of sowing dates determined by observations over a series of years.

Moreover, in years of moderate infestation, when the wave of infestation is on the up-grade, but because of lack of parasites considerable menace exists, it will be extremely difficult, if not entirely impracticable, to find enough flaxseeds for examination. In actual practice, even in years of heavy infestation, when parasitism has been heavy but not sufficiently so to remove the fly menace, considerable difficulty has been met in getting satisfactory records, and in some cases it was not at all practical to get them. Entirely successful cases of emergence have been obtained, but these almost without exception, have occurred in regular years when the emergence fitted in quite nicely with the established and set series of dates.

It would seem, therefore, that the practical application of the emergence cage to Hessian-fly investigations is confined principally to life history and control studies of other than a predictive nature.

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THE REACTION OF CERTAIN GRASSES TO CHINCH-BUG ATTACK¹

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INTRODUCTION

The bunch grasses of the prairies in the natural state are known to be the hibernating place of the chinch bug (*Blissus leucopterus* Say), which migrates from these grasses to cultivated members of the grass family (Poaceae), such as wheat, corn, and the sorghums. It is of special interest, therefore, to know what occurs when other grasses are attacked, both the effect on the grass, and, either because of slight injury or recovery, the extent to which it might serve as a hibernating medium. Chinch bugs have been recorded as attacking a number of grasses, but there seems to be no extended discussion in the literature on the importance of the grass family, other than cultivated crops, as food for chinch bugs. Webster² and Horton and Satterthwait³ list a number of wild or native grasses attacked by this species. The present paper, besides greatly extending the previous lists of known food plants, seeks to show the degree of injury to the host plants and their ability to recover from injury.

During the summer of 1924, an opportunity occurred to record the data assembled here. A special grass garden of the Department of Botany and Plant Pathology of the Kansas Agricultural Experiment Station, containing about 100 grasses, used primarily for studies on the overwintering of leaf-rust of wheat, was located at the station on land adjoining wheat plots of a winter-wheat nursery in which there appeared large numbers of chinch bugs. The wheat was cut about July 1, and the bugs migrated to the adjacent grass garden. By July 8, injury to the various grasses was manifested in such varying degrees that it was deemed advisable to record the differences. A few weeks later, the chinch bugs left the grasses and journeyed to neighboring corn and sorghum. Rainfall was abundant after August 1, and some plants began to show signs of recovery. By August 18 the differences were so marked that notes on the ability to recover were taken.

CULTURE OF GRASSES

The grass garden has been maintained for a number of years and many of the rows consisted of large, robust plants at least two years of age. In maintaining this garden those grasses which survive

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² WEBSTER, F. M. THE CHINCH BUG. U. S. Dept. Agr. Farmers' Bul. 657, p. 7, 1915.

³ HORTON, J. R., and SATTERTHWAIT, A. F. THE CHINCH BUG AND ITS CONTROL. U. S. Dept. Agr. Farmers' Bul. 1223, p. 35, 1922.

the winter are retained for further study, while those perennials which winterkill and all annuals are replanted each spring. To insure satisfactory growth it has been found necessary to plant the seed in small flowerpots in the greenhouse about March 1. This gives the seedlings a good start. After danger of severe frost injury is past, which is usually about April 1, they are transplanted to rows in the garden. This method of culture gives most of the grasses an excellent early-season growth. There are usually a few instances in which this treatment produces either extremely early or very late maturity. Such cases are rare, however, and therefore of no great significance. Then the grasses are transplanted to the garden rows, the plants are knocked from the pots and divided into small tufts, care being exercised to avoid any injury to the roots. The tufts then are carefully set in shallow trenches, and the soil firmed around them. The plants are placed in rows 5 feet long and 2 feet apart. This usually gives a considerable number of plants for observation, and prevents serious competition between species in adjacent rows.

The grasses made an exceptionally good start in the spring of 1924. Most of the rows had excellent stands. Although only a few plants were obtained in some rows, these were always normal. The early spring conditions so greatly favored the growth of grasses that most species had made a large amount of leaf growth before the chinch-bug migration from the wheat to the grasses had begun.

FACTORS CONSIDERED

ANNUAL OR PERENNIAL

One of the first plant characters to be considered in a study of this nature is the longevity of the plant. A short-lived annual might reasonably be expected to make an earlier and more vigorous growth than longer-lived perennials. In making observations on the chinch-bug injury to the grasses, it was noted that perennial species consistently exhibited much more resistance to attack than annuals. This may be due in part to the fact that most of the perennials had harsh leathery tissues, while most annuals are characterized by tender tissues which often are thin and sometimes rather flaccid. The chinch bugs attacked the annuals first and within a few days killed most of them.

It is realized that some plants are listed as annuals which, under other conditions, may be perennial, and vice versa. In some years at Manhattan certain species may survive the winter, while in others the same species may be completely killed. The habit given in this paper refers, therefore, to the behavior of the species under Kansas conditions.

NATIVE OR INTRODUCED

In taking notes upon the amount of chinch-bug injury in the grass garden, it was observed immediately that species indigenous to Kansas exhibited striking resistance. It was further noted that the species native and common to that part of the State in which chinch-bug injury to cultivated crops most commonly occurs showed the greatest tolerance to attack. Furthermore, those same grasses comprise by far the greater percentage of the plants found in pastures, meadows, sod roadsides, and undisturbed fence rows. These are

the plants in which the chinch bugs overwinter in their normal habitat. On the other hand, grasses not native to Kansas were usually found to be severely injured, although in many cases they possess plant characters which were commonly found associated with resistance in indigenous species. Different species of the same genus, which vary primarily only in their natural range, also showed marked differences in their reaction to chinch bugs. Therefore, in this study the terms "native or introduced" are not used in the usual sense, but refer only to whether the species is native to Kansas or not.

CHARACTER OF TISSUES

A wide difference was noted in the character of the plant tissues of different species. In general, however, the plants could be grouped into three classes: (1) Those having noticeably harsh, tough, or leathery tissues; (2) those with soft or tender tissues; and (3) those with tissues intermediate between the two extremes. In Table I these characters are indicated in the column headed "character of tissues" as harsh, tender, and intermediate. Grasses with harsh tissues are harsh to the touch, are glabrous or only slightly pubescent, and usually are rigidly erect as to leaf and culm characters. Harshness in the grasses has been ascribed by some writers to a high percentage of silicon in the vegetative parts. Tender-tissued plants are pubescent, as a rule, though not always so. The leaves are often thin and soft to the touch and are commonly drooping, thus giving a somewhat flaccid appearance to the plants. This is particularly true of the annual species of *Bromus*. The third group possesses tissues intermediate in character. Plants of this group may be pubescent or glabrous, but the tissues are leathery rather than harsh or tender.

It is interesting to note that such species as *Sporobolus cryptandrus*, *S. asper*, *Sorghastrum nutans*, *Eragrostis trichodes*, *Elymus virginicus*, *E. canadensis*, *Triodia flava*, *Bouteloua oligostachya*, *B. curtipendula*, and *Andropogon scoparius*, which exhibited the greatest resistance to chinch-bug attack, are all perennial native species with harsh tissues. These are also the commonest roadside, pasture, and prairie grasses in the vicinity of Manhattan.

TIME OF MATURITY

The time of maturity refers to the period during which each species has been observed to flower and mature seed at Manhattan. In a number of cases, however, where new species were being grown for the first time and no first-hand information on this point was available, it was obtained from published floras. A few of the species matured before the chinch-bug attack, but the majority would not have matured until afterward. Plants of related species in various stages of growth were available for observation. In a few cases, plants of the same species of various ages were available. *Hystrix hystrix* was a case in point. The plants in row 35 were large robust plants, two years old, while those in row 73 were young plants grown from the seed in the spring of 1924. In this instance the older plants were attacked and killed by the bugs as readily as the young plants. In fact, no relation was noted between the stage of maturity of the plants and their reaction to chinch bugs. It is, however,

important to have information on the maturity of species from the standpoint of overwintering and early spring feeding of the bugs. Those species which mature extremely early usually are blown away before fall and therefore would afford very little winter protection, while the later-maturing species retain their dead stems and leaves throughout the winter, making excellent chinch-bug harbors. Most of the common native perennial grasses found in the vicinity of Manhattan are of that nature.

TYPE OF PLANT GROWTH

The characteristic types of growth exhibited by the plants under culture are noted as turf-forming, dispersed, tufted, and bunch. The terms "turf-forming" and "bunch" are self explanatory. In those species of dispersed habit, the plants usually are found singly or scattered over a considerable area. Such plants usually have only a few culms and do not form definite tufts or clumps, although there may be a large number of plants in a small area. The plants occurring in tufts are usually low-growing species, such as species of *Bouteloua*; while bunch types are usually large and coarse-stemmed, such as species of *Andropogon*, *Sorghastrum nutans*, and *Triodia flava*.

HABITAT

Information concerning the habitats of the various species was obtained from a number of sources. That given for the species indigenous to Kansas was taken from the junior author's notes and experience with those species. For the exotic species, knowledge of the habitat was obtained from various publications on the flora of the United States and Europe. Wherever possible the kind of place in which the species occurs most commonly is referred to, in this paper, as its habitat. It is of considerable interest to note that those grasses which were the most resistant to chinch-bug attack were those which occur most commonly as roadside or fence-row grasses, and comprise most of the pasture and prairie grasses indigenous to Kansas. Two roadside and fence-row conditions obtain in Kansas, one in which the original sod has not been disturbed, and in which the native perennial prairie grasses predominate, and another in which the soil has been disturbed and annual species such as *Bromus tectorum*, *B. arvensis*, *Hordeum pusillum*, *H. jubatum*, and annual species of *Fragrostis* predominate.

There are a number of species which are listed as occurring in cultivated fields. Such grasses as *Bromus tectorum*, *B. arvensis*, *Hordeum jubatum*, and species of *Lolium* often appear in wheat fields and sometimes become serious weeds. Within the last three years *Aegilops cylindrica* (*Triticum cylindricum*) has appeared as a noxious weed in wheat fields and disturbed roadsides and fence-rows in central Kansas. This species is of interest from the standpoint of chinch bugs, since it is a winter annual like wheat, often occurs as a volunteer weed in the late summer, and is so closely related to wheat as to hybridize with it.

CHINCH-BUG INJURY

Within a few days after the adjacent wheat was harvested most of the rows of grasses were literally swarming with chinch bugs in all instars of nymphal development, as well as thousands of adult

insects. Many of the different grasses soon were killed or severely injured, while others showed varying degrees of resistance. Notes regarding these differences were taken on July 8. Since such data, at best, can be only relative, it was thought advisable to list the degree of injury in as few categories as possible. Accordingly the following degrees of injury were considered. If a row of grass was entirely dead it was noted as *killed*; more than half dead was considered *severe*; if only half dead it was regarded as *moderate*; and when only a few plants were injured it was listed as *slight*.

PERCENTAGE OF RECOVERY

The observations on the ability of the various species of grasses to recover from injury were made on August 18. Very favorable growing weather had prevailed between this date and the time when the insects attacked the plants. By this date, also, most of the chinch bugs had become mature and had flown to neighboring corn and sorghum fields.

In estimating the percentage of recovery, rows in which no live plants were present were listed as zero, meaning no recovery; those in which only one or two plants were alive were marked 1 per cent, and so on, until those rows in which there seemed to be complete recovery were noted as 100 per cent. Recovery data for the few rows in which there was no injury are not given in Table I.

PRESENTATION OF DATA

To the data on the amount of injury and the degree of recovery have been added the morphological and ecological features of the various grasses discussed in the previous paragraphs and these have been summarized in Table I.

DISCUSSION

From Table I it seems strikingly apparent that the character of the vegetation and the circumstance of whether the species of grass is native or introduced, have considerable to do with the ability of the plant to withstand chinch-bug attacks. Those grasses having harsh tissues seem, in most cases, to be especially resistant, if they are native grasses. For example, such a plant as *Andropogon scoparius* (row 3) has harsh tissues and is a native perennial. It is well known that it affords the chinch bug protection during the winter, and, in the case noted, no injury was apparent, although the plants were literally covered with chinch bugs. In the majority of instances of those grasses termed "harsh" in which chinch-bug injury was severe and recovery poor, the species were introduced ones.

It will be observed further that those grasses listed as "intermediate" in regard to their vegetative characters showed varying degrees of resistance. Some, such as *Festuca ovina* (row 99), showed only slight injury, and with marked ability to recover. Others, like *Bromus secalinus* (row 57), showed only moderate injury and mediocre ability to recover. Still others were severely damaged, e. g., *Elymus glaucus* (row 13), and were unable to recover.

TABLE I.—The reaction to chinch-bug attack of certain grasses in the grass garden, Kansas Agricultural Experiment Station, Manhattan, Kans.

Row	Scientific name	Common name	Annual or perennial	Native or introduced	Character of tissues	Time of maturity	Type of growth	Habitat	Chinch-bug injury	Per cent of recovery
1	<i>Bromus patulus</i>	Spreading brome grass	A	I	Tender	July-Aug.	Tufted	Waste places	Killed	0
2	<i>Anthoxanthum odoratum</i>	Sweet vernal grass	P	I	Harsh	June-July	do	Meadows	Slight	70
3	<i>Andropogon scoparius</i>	Little bluestem	P	N	do	Aug.-Sept.	Bunch	Prairies and sod roadsides	None	0
4	<i>Andropogon lagroides</i>	P	N	do	July-Sept.	do	do	Slight	100
5	<i>Bromus sp. (octoensis?)</i>	A	I	Tender	May-July	Tufted	Waste places	Killed	0
6	<i>Bromus arvensis</i>	False cheat	A	N	do	do	do	Disturbed places, roadsides	Moderate	1
7	<i>Bromus lanuginosus</i>	A	I	do	June-July	do	Waste places	Severe	0
8	<i>Hordeum bulbosum</i>	A	I	Harsh	May-June	Dispersed	do	do	10
9	<i>Bromus arvensis</i>	False cheat	A	N	Tender	May-July	Tufted	Disturbed places, roadsides	Moderate	0
10	<i>Bromus erectus</i>	Upright brome grass	A	I	Harsh	July-Aug.	Dispersed	Waste places	Slight	100
11	<i>Agropyron lanceolatum</i>	P	I	do	July-Sept.	do	Sod land roadsides	Severe	1
12	<i>Bromus laevipes</i>	A	I	Tender	June-July	Tufted	Waste places	do	0
13	<i>Elymus glaucus</i>	Smooth wild rye	P	I	Intermediate	June-Aug.	Bunch	do	do	0
14	<i>Bromus pumellianus</i>	P	I	Harsh	do	do	do	Slight	100
15	<i>Hordeum maritimum</i>	Seaside barley	A	I	do	June-July	Tufted	Sand shores, waste places	(*)	0
16	<i>Elymus caput-medusae</i>	A	I	Intermediate	July-Sept.	Bunch	Waste places	Severe	20
17	<i>Lolium subulatum</i>	A	I	do	May-June	Dispersed	Meadows	do	0
18	<i>Bromus breviaristatus</i>	Short-awned cheat	A or P	I	Harsh	July-Aug.	do	Waste places	Moderate	3
19	<i>Bromus sitchensis</i>	P	I	do	July-Sept.	Bunch	do	Slight	100
20	<i>Elymus striatus</i>	Slender wild rye	P	I	do	June-July	do	Shady places	Severe	1
21	<i>Elymus condensatus</i>	P	I	do	July-Aug.	do	Waste places	Moderate	40
22	<i>Bromus pratensis</i>	A	I	Tender	do	Tufted	do	do	20
23	<i>Bromus rigidus</i>	Rigid brome grass	A	I	do	do	do	Waste places	Killed	0
24	<i>Paspalum floridanum</i>	Florida paspalum	P	I	Harsh	Aug.-Sept.	Bunch	do	Slight	100
25	<i>Lolium temulentum</i>	Darnel	A	I	do	May-June	Dispersed	Cultivated fields waste places	(*)	0
26	<i>Phalaris minor</i>	Small canary grass	A	I	Tender	June-Aug.	do	do	Killed	0
27	<i>Sporobolus cryptandrus</i>	Sand drop seed	P	I	Harsh	Aug.-Sept.	Tufted	Sod roadsides	Slight	95
28	<i>Sporobolus airoides</i>	Hair-grass drop seed	P	I	do	do	Bunch	Prairie, sod roadsides	Moderate	100
29	<i>Sporobolus asper</i>	Long leaved rush grass	P	N	do	do	do	do	None	0
30	<i>Sorghastrum nutans</i>	Indian grass	P	N	do	do	do	do	do	0
31	<i>Hordeum caespitosum</i>	A	I	Tender	May-June	Dispersed	Waste places	(*)	0
32	<i>Bromus tectorum nudus</i>	P	I	do	June-July	do	Disturbed places	Killed	0
33	<i>Agropyron violaceum</i>	A	I	Harsh	July-Aug.	Turf	Roadsides	Severe	0
34	<i>Hystrix hystrix</i>	Bottle brush grass	P	I	do	June-July	Bunch	Sod places	Killed	0
35	<i>Oryzopsis miliacea</i>	Mountain rice	A	I	do	July-Sept.	Dispersed	High altitudes	(*)	0
36	<i>Oryzopsis miliacea</i>	A	I	do	Aug.-Sept.	Bunch	Meadows, roadsides	Slight	100
37	<i>Triodia flava</i>	Purple top	P	N	do	do	do	do	do	0

No.	Plant	Locality	Time	Condition	Notes	Remarks
38	<i>Triodia albescens</i>	Reed canary grass	June-Aug	Intermediate	do.	100
39	<i>Phalaris arundinacea</i>	Bromus polyanthus	July-Aug	Intermediate	do.	100
40	<i>Bromus polyanthus</i>	Great brome grass	do.	do.	do.	95
41	<i>Bromus maximus</i> (B. rigidus)	Great brome grass	do.	do.	do.	0
42	<i>Hordeum murinum</i>	Wall barley	June-July	Intermediate	do.	0
43	<i>Festuca rubra</i>	Red fescue	May-June	Harsh	do.	100
44	<i>Arrhenatherum elatius</i>	Tall oats grass	June-Aug	do.	do.	100
45	<i>Eragrostis trichodes</i>	Blow-out grass	July-Sept.	do.	do.	90
46	<i>Eragrostis pilosa</i>	Small tufted love grass	do.	do.	do.	100
47	<i>Eragrostis lugens</i>	Small tufted love grass	Aug-Sept.	do.	do.	0
48	<i>Hordeum gussoneanum</i>	Barren brome grass	June-July	Tender	do.	0
49	<i>Bromus sterilis</i>	Barren brome grass	do.	do.	do.	0
50	<i>Bromus villosus</i> (B. rigidus)	Barren brome grass	do.	do.	do.	0
51	<i>Bromus rubens</i>	Darnel	do.	do.	do.	0
52	<i>Lolium temulentum</i>	Squirrel-tail grass	do.	do.	do.	0
53	<i>Hordeum jubatum</i>	Rescue grass	do.	do.	do.	0
54	<i>Bromus unioloides</i>	Rescue grass	do.	do.	do.	0
55	<i>Bromus secalinus</i>	Downy brome grass	do.	do.	do.	0
56	<i>Bromus tectorum</i>	Hungarian brome grass	do.	do.	do.	0
57	<i>Bromus inermis</i>	Meadow barley	do.	do.	do.	0
58	<i>Hordeum nodosum</i>	Coast wheat grass	do.	do.	do.	0
59	<i>Agropyron pungens</i>	Spike grass	do.	do.	do.	0
60	<i>Leptochloa fascicularis</i>	Virginia wild rye	do.	do.	do.	0
61	<i>Elymus virginicus</i>	Kalm's cheat	do.	do.	do.	0
62	<i>Bromus kalmii</i>	Orchard grass	do.	do.	do.	0
63	<i>Dactylis glomerata</i>	Awned wheat grass	do.	do.	do.	0
64	<i>Agropyron caninum</i>	Meadow fescue	do.	do.	do.	0
65	<i>Festuca elatior</i>	Feather-bunch grass	do.	do.	do.	0
66	<i>Stipa viridula</i>	Blue grama grass	do.	do.	do.	0
67	<i>Bouteloua gracilis</i>	Canada lyme grass	do.	do.	do.	0
68	<i>Elymus canadensis</i>	Carolina canary grass	do.	do.	do.	0
69	<i>Phalaris caroliniana</i>	Bottle-brush grass	do.	do.	do.	0
70	<i>Hystrix patula</i> (H. hystrix)	Lolium sp. (argenteum)	do.	do.	do.	0
71	<i>Festuca sp.</i>	Small bent grass	do.	do.	do.	0
72	<i>Agrostis verticillata</i>	Barren fescue	do.	do.	do.	0
73	<i>Festuca bromoides</i>	Straw-colored paspalum	do.	do.	do.	0
74	<i>Paspalum setaceum</i>	Prairie chloris	do.	do.	do.	0
75	<i>Chloris verticillata</i>	Wavy hair grass	do.	do.	do.	0
76	<i>Deschampsia flexuosa</i>	Side-oats grama	do.	do.	do.	0
77	<i>Bouteloua curtipendula</i>	Upland bent grass	do.	do.	do.	0
78	<i>Agrostis perennans</i>	Marsh foxtail	do.	do.	do.	0
79	<i>Alopecurus geniculatus</i>	Marsh foxtail	do.	do.	do.	0

^a Died from other causes before bug attack.^b Matured before bug attack.

^c These occur rarely in southwestern Kansas; have been introduced into the Manhattan locality.

^d In rows Nos. 34, 44, 55, 67, 91, 92, and 95, no stand was obtained.

• *Hystrix hystrix* = *Hystrix patula*. Row 35 received from Indiana (2-year-old). Row 73 received from Idaho (seedlings). In rows nos. 31, 33, 35, 37, 39, 41, 43, and 45, no stand was obtained.

s Awned type; typical *L. temulentum* is awnless.

TABLE I.—The reaction to chinch-bug attack of certain grasses in the grass garden, Kansas Agricultural Experiment Station, Manhattan, Kans.—
Continued

Row	Scientific name	Common name	Annual or perennial	Native or introduced	Character of tissues	Time of maturity	Type of growth	Habitat	Chinch-bug injury	Per cent of recovery
84	<i>Festuca tenuifolia</i>	Gramma grass.....	A or P	I	Intermediate.	July-Sept.	Tufted.....	Prairies, sod roadsides.....	Severe.....	4
85	<i>Bouteloua oligostachya</i> (B. gracilis).		P	N	Harsh.....		do.....		Slight.....	100
86	<i>Festuca myuros</i>	Rats-tail fescue.....	P	I	do.....	June-July.....	do.....	Waste places.....	Killed.....	0
87	<i>Schedonnardus paniculatus</i>	Texas windmill grass.....	P	N	do.....	do.....	do.....	Prairies, sod roadsides.....	Severe.....	100
88	<i>Sphenopholus obtusata</i>		A	N	Tender.....	May-June.....	Dispersed.....	Meadows.....	Slight.....	10
89	<i>Eragrostis lugens</i>		P	I	Intermediate.....	July-Aug.....	Bunch.....	Prairies.....	Severe.....	15
90	<i>Eragrostis secundiflora</i>	Clustered love grass.....	A	N	do.....	Aug.-Sept.....	Dispersed.....	Waste places, dry soil.....	Killed.....	0
93	<i>Bouteloua oligostachya</i> (B. gracilis).	Gramma grass.....	P	N	Harsh.....	July-Aug.....	Tufted.....	Prairies and sod roadsides.....	Slight.....	100
94	<i>Sitanion hystrix</i>		P	I	do.....	do.....	do.....	Prairies.....	Killed.....	0
96	<i>Anthoxanthum odoratum</i>	Sweet vernal grass.....	P	I	do.....	June-July.....	do.....	Meadows.....	Moderate.....	95
97	<i>Bromus hordeaceus</i>		A	I	Tender.....	July-Aug.....	Dispersed.....	Waste places.....	Killed.....	0
98	<i>Pennisetum japonicum</i>		P	I	Harsh.....	Aug.-Sept.....	Bunch.....	Ornamental.....	Moderate.....	10
99	<i>Festuca ovina</i>	Sheep fescue.....	P	I	Intermediate.....	June-July.....	Turf.....	Cultivated.....	Slight.....	100
100	<i>Aegilops cylindrica</i> (<i>Triticum cylindricum</i>).		W ^a	N	Tender.....	do.....	Dispersed.....	Cultivated, fields.....	Killed.....	0

^a These occur rarely in southwestern Kansas; have been introduced into the Manhattan locality.

^b Winter annual.

Among the plants with tender tissues, there was only one species, *Sphenopholus obtusata* (row 88), which showed but slight damage. A few species, for example *Bromus arvensis* (row 6), showed only moderate injury. The majority of tender grasses were either severely injured or completely killed.

One instance is noted, in the case of *Hystrix hystrix* (row 35), in which the age of the grass seemed to make no marked difference in the ability of the plant to withstand injury. Row 35 was a 2-year old stand of *Hystrix hystrix* seed of which originally came from Indiana. Row 73 was composed of seedling plants of the same species, seed of which was received from Idaho under the name of *Hystrix patula* and so listed in the plots. Resistance was no more noticeable in the 2-year-old strain than in the 1-year-old strain, for both were killed.

No correlation is apparent between habit of growth (that is, whether the plants are tufted or bunched) and the degree of injury and recovery. Bunch types were alike slightly injured or killed, and the same can be said of the turf, tufted, and dispersed types. The date of maturity of the various species has been listed herein because it was noted that several species had matured and produced seed before the migration of the chinch bugs from the wheat to the grasses. This fact may have an important bearing in badly-infested areas where the production of an early seed crop is desired.

No correlation is seen between habitat and degree of injury, although it is important to note that some grasses which occur as pests in cultivated fields, such as *Aegilops cylindrica* (row 100), may be killed by the bugs. Others which occur in plowed fields, such as *Lolium temulentum* (row 53), may mature and produce seed before the insect attack begins; while grasses like *Festuca elatior* (row 68), also found in cultivated areas, may show only moderate injury and marked recuperative powers.

SUMMARY

During the season of 1924, the grasses in the grass garden of the Kansas Agricultural Experiment Station were attacked by large numbers of chinch bugs which migrated from adjacent wheat fields after harvest. The different species showed different degrees of resistance to injury, and later some of them exhibited marked ability to recover from the attack. It was apparent that native perennial species with harsh tissues were able to survive chinch-bug injury and showed the most marked ability to recover. These grasses comprise about 80 per cent of the native prairie grasses of Kansas.

Besides the data regarding the resistance and recovery of the grasses, a large number of species of grass not heretofore recorded as host plants of the chinch bug are listed.



DIFFERENCE IN INTERNODE LENGTHS BETWEEN, AND EFFECT OF VARIATIONS IN LIGHT DURATION UPON, SEEDLINGS OF ANNUAL AND BIENNIAL WHITE SWEET CLOVER¹

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HISTORICAL DATA

The effect of environment on the growth of plants has probably been studied more extensively by Klebs than by any other worker. He studied not only the effect of various salts, of temperature and moisture, but in his later papers touched on the effect of illumination for different periods on the blossoming of *Sempervivum*.² In this he showed that the blossoming of *Sempervivum albidum* and *S. funkii* could be affected by varying the hours of illumination to which rosettes were subjected while flower buds were forming. Later Garner and Allard³ studied the effect of varying periods of illumination on a number of plants and established the fact that in many cases at least the blossoming of a species depends on the hours of illumination.⁴

In a second paper,⁵ Garner and Allard touch on the apogeotropic response to photoperiodicity. They found that increasing the length of day increased the total stem length of *Amaranthus hispidus* and other plants, but that the reverse was true of sorghum and certain small grains. They do not state whether this lengthening of the axis was in the form of increase in the number of internodes or increase in the length of each internode. In *Melilotus alba*, the increased length was due wholly to an increase in the length of each internode.

The experiments just referred to showed, as did those of Oakley and Westover, that the length of the period of illumination may be expected to influence not only the blossoming but also the vegetative growth of the plant. Under a short day the internodes were shortened, while under a long day they were very greatly elongated. The degree to which the seedlings were affected varied somewhat with the variety. Doubtless other plants will be found to react in a similar manner.

EXPERIMENTAL DATA

In August, 1921, seeds of annual and of biennial white sweet clover were planted in parallel rows at the Arlington Experiment Farm, Rosslyn, Va. On October 4, 1921, it was noted that the annual plants were taller than those of the biennial, though the number of

¹ Received for publication October 16, 1924; issued October, 1925.

² KLEBS, G. UEBER DIE BLÜTENBILDUNG VON SEMPERVIVUM. Flora (N. F. 11/12) 111/112: 128-151, illus. 1918.

³ GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

⁴ As Garner and Allard have already adequately reviewed the literature in their two papers, no attempt to do so is made in this paper.

⁵ GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

leaves on both was about the same. The difference between them was evidently due to the greater internode length of the former (pl. 1). It was at once suggested that this difference might be utilized as a means of identifying the seed of the annual then coming into prominence and the seed of which is indistinguishable from that of the biennial.

On October 11, when 60 days old, these seedlings were taken up and measured from: (1) Cotyledons to tip of the longest leaf, and (2) cotyledons to growing point of the stem. Naturally, the exact location of the growing point could not be ascertained without elaborate dissection in each case. However, by careful observation, its position could be determined very closely, and the writer is confident that the error will not exceed ± 0.5 mm.

In the seedlings of *Melilotus*, as in *Trifolium*, *Medicago*, and many other legumes, the first leaf is unifoliolate (pl. 1, *a*) and all subsequent leaves trifoliolate. Counting the unifoliolate leaf as one, the number of expanded or nearly expanded leaves is equal to the number of internodes above the cotyledons, though the upper internode may not yet have reached full length. By noting the number of expanded or nearly expanded trifoliolate leaves, a means is afforded for comparing seedlings of equal development.

In the work here reported, all seedlings taken up on a given date were separated into groups, each group containing those plants with the same number of expanded, or nearly expanded, trifoliolate leaves. The last internode lengthens as the upper leaf expands, and a part of the irregularities in the measurements recorded in Table I is due to unequal maturity of the upper internodes. The cotyledons⁶ remain attached for many days or weeks and afford a convenient and fixed joint from which to measure (pl 1, *b*). The measurement from cotyledons to tip of longest leaf was found to be unsatisfactory, because of great variations in petiole lengths, and this measurement was abandoned. In measurements made subsequent to those recorded in Table I the length from cotyledons to first (unifoliolate) leaf, and the length from cotyledons to growing point were made. In all, 4,610 seedlings were measured, involving more than 9,000 measurements.⁷

TABLE I.—Average stem and internode lengths in millimeters of seedlings measured October 11, 1921, 60 days after date of seeding

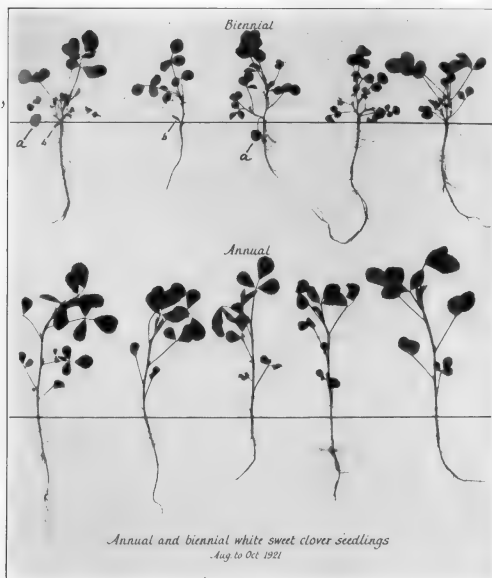
	Annual			Biennial		
	3-leaved ^a	4-leaved	5-leaved	3-leaved	4-leaved	5-leaved
Average stem length.....	22.4	35.8	45.55	7	10.8	11.1
Average internode length.....	5.6	5.16	7.6	1.75	2.16	1.85

^a The number of internodes was one more than the number of trifoliolate leaves.

It will be seen from Table I that the length of the internodes in the annual was two, three, or more times as great as in the biennial (pl. 1).

These facts were reported at a meeting of the Association of Official Seed Analysts of North America, held in July, 1923.⁸

⁶ In all plates showing mounted specimens, the plants have been so mounted that the cotyledons, or the point where they were attached, are on the heavy black line.
⁷ The writer's thanks are due to L. W. Kephart for assistance in making these measurements.
⁸ ASSOCIATION OF OFFICIAL SEED ANALYSTS. PROCEEDINGS OF THE FOURTEENTH AND FIFTEENTH ANNUAL MEETINGS. 148 p. 1923



Showing difference in stem and root development between biennial and annual white sweet clover grown outdoors August 10 to October 4, 1921

As a result of this first observation seeds of annual and biennial white sweet clover were sown in the greenhouse on December 24, 1921, and the flats subjected to various periods of illumination: (1) Normal day for that time of year, about 9½ hours;⁹ (2) short day, from 9 a. m. to 4 p. m.; (3) long day, from daylight to dark, with illumination, from twilight until 11 p. m., by three 200-Watt electric lamps hung about 2 feet above the flats. The temperature of the greenhouse was 50 to 60° F. The rise of temperature caused by the electric lights was inconsiderable, as was that in the dark chamber.¹⁰ Under these conditions the following six lots of seed were tested:

- 2066. Grundy County biennial, an early dwarf form.
- 2140. Commercial biennial.
- 03464. Arctic biennial, an early dwarf form.
- 2143. Mixed annual.
- 12055. A late annual selection.
- 12060. An early annual selection.

The seedlings were measured 51 days after sowing. The measurements are given in Table II.

TABLE II.—Average length of the first internode, average stem length, and average internode length of seedlings, 51 days from sowing on December 24, 1921

[Measurements in millimeters]

		3-leaved						4-leaved					
		Biennial			Annual			Biennial			Annual		
		2066	2140	03464	2143	12055	12060	2066	2140	03464	2143	12055	12060
N	I'	8.5	12.3	11.6	14.4	19	16.9	10.9	15	13.9	14.3	19.6	17
	S	14.3	22.4	17	25	36	30	22	29.3	26	33.5	45.5	38.1
	I	1.9	3.4	1.8	3.5	5.7	4.4	2.8	3.6	3	4.8	6.5	5.4
S	I'	3	5	8.5	6.2	6	5.3	4.8	5.5	8.6	6.8	7.1	5.5
	S	4.45	7.35	18.3	10	10	8	7.6	9.9	18.8	12.5	11.8	8.3
	I	.5	.8	3.3	1.3	1.3	.9	.7	1.1	2.5	1.4	1.2	.7
L	I'	13.3	18.8	23.6	21	23.7	20.4	15.4	18.3	28	23.2	23.6	22.2
	S	28	40	43.5	44.3	45	40.9	40.2	48.5	57	58.8	58	55.7
	I	4.9	7.1	6.6	7.4	7.1	6.6	6.2	7.5	7.2	8.9	6.1	8.4

⁹ N=normal day. S=short day. L=long day. I'=length first internode; cotyledons to unifoliate leaf. S=stem length; cotyledons to growing point. I=average length all internodes above the first. This figure is found by dividing stem length from unifoliate leaf to growing point by the number of trifoliate leaves.

It will be noted that the first internode is always considerably longer than the others. For example, in Table II, if the length of the first internode of 2066, biennial, for normal day, is deducted from the total stem length, the length above the unifoliate leaf is 5.75 millimeters, or an average of slightly less than 2 millimeters per internode. In 2143, annual, this length is 3.5 millimeters. In the three and four leaved seedlings, the length of the first internode, under normal day, equals or exceeds that of the entire stem above the unifoliate leaf, but in older seedlings this relation changes. See Table III, where in the normal day columns for the seven-leaved seedlings, the average length of each internode above the unifoliate leaf is but little less than that of the first internode.

⁹ Data from GARNER, W. W., BACON, C. W., and ALLARD, H. A. PHOTOPERIODISM IN RELATION TO HYDROGEN-ION CONCENTRATION OF THE CELL SAP AND THE CARBOHYDRATE CONTENT OF THE PLANT. Jour. Agr. Research 27: 119-156, illus. 1924.
¹⁰ OAKLEY, R. A., and WESTOVER, H. L. EFFECT OF THE LENGTH OF DAY ON SEEDLINGS OF ALFALFA VARIETIES AND THE POSSIBILITY OF UTILIZING THIS AS A PRACTICAL MEANS OF IDENTIFICATION. Jour. Agr. Research 21: 599-608, illus. 1921.

TABLE III.—Average length of first internode, average stem length, and average internode length of seedlings, 63 days from sowing, January 12, 1922

[Measurements in millimeters. Lettering same as for Table II]

	5-leaved						6-leaved						7-leaved					
	Biennial			Annua			Biennial			Annual			Biennial			Annual		
	2066	2140	03464	2143	12055	12060	2066	2140	03464	2143	12055	12060	2066	2140	03464	2143	12055	12060
N { I'...	None.	5.9	4.5	7	11.1	9.2	4.3	5.8	6	8.4	11.6	9.9	4	6.4	5.5	None.	11	9.8
N { S...	None.	25	18.2	26	45	45.8	16.5	30	23.1	43.8	55.8	55.9	25	49.6	24.7	None.	72.5	72.4
N { I...	None.	3.8	2.7	3.8	4.8	7.3	2	4	2.8	5.2	7.4	7.7	3	6.1	2.7	None.	8.8	8.9
N { I'...	2.7	3.1	4.6	5	None.	5.3	2.2	3.1	5.5	5	4.2	5.2	2	3.1	4.6	4.2	4	5.4
N { S...	9.1	11.5	15.7	21.4	None.	35.8	9	13.3	20.5	20.5	18	42.7	9.7	13.5	19	22	18.4	52.5
N { I...	1.3	1.7	2.2	3.3	None.	6.1	1.1	1.7	2.5	2.6	2.3	6.2	1.1	1.5	2	2.5	2.1	6.7
N { I'...	14.7	14.2	13.7	16.5	19	16	13.7	16	17.7	16	19.7	17.8	15	17.1	17.7	18	21.3	17.5
N { S...	73.4	72.6	73	76	93	89.5	97.3	106.6	120.5	96.4	121.2	117.8	120.7	142.3	120.5	135	157.6	146
N { I...	11.7	11.7	11.9	11.9	15	14.7	13.9	15.1	17.1	13.4	16.9	17	15.1	17.9	14.7	17	19.5	18.4

As shown in Table II, the internode length of the annual forms was, under the normal length of day, markedly greater than that of the biennial forms (pl. 2, top row), though the difference was ont as great as it had been in seedlings grown out of doors in August-October.

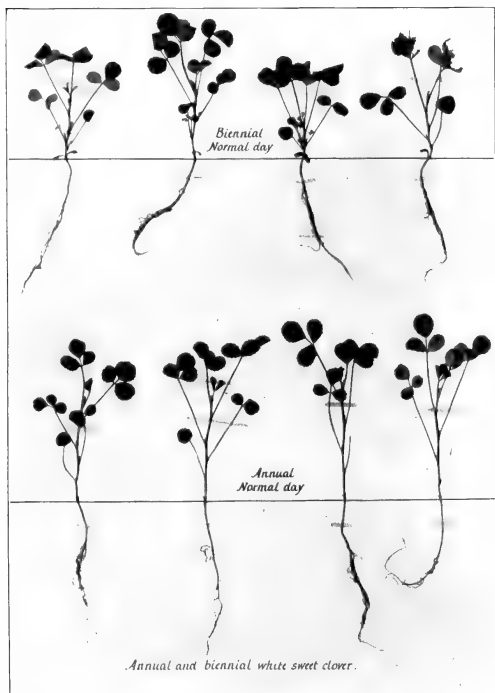
This fact is also brought out in Plate 3, which should be compared with Plate 1. The effect of decreased illumination was to shorten the internodes, both first and subsequent ones, but this shortening was, in the Arctic variety, 03464, confined to the first internode and was not shown by the subsequent internodes. Increased illumination was marked by an elongation of all internodes. For the plants recorded in Table II, and leaving out the Arctic as peculiar, the ratio of average length of internodes between annual and biennial is: For three-leaved seedlings, normal day 1 to 0.58, short day 1 to 0.54, and long day 1 to 0.86; for four-leaved, normal day 1 to 0.57, short day 1 to 0.8, and long day 1 to 0.87.

Under the long day the difference between the internode lengths of annual and biennial has been decreased, the biennials responding relatively more to the long day than the annuals. The relative size of the first internode of annual and biennial has, however, remained fairly constant under long and short days, showing that the effect of illumination has been felt mostly in the internodes after the formation of the first leaf. For the first internode the ratios of annual to biennial are as follows (Table II): Three-leaved, normal day 1 to 0.61, short day 1 to 0.69, long day 1 to 0.73; for four-leaved, normal day 1 to 0.76, short day 1 to 0.78, long day 1 to 0.73.

On January 12, 1922, a second set of the same numbers was sown and allowed to grow until March 16, 63 days after seeding. These seedlings were therefore older than those in the first set, and those under normal day had also had more hours of sunlight than those in the first set. In a few cases the number of seedlings of the seven and eight leaved sizes fell below 10 for each measurement, so these figures have less value than those in which more seedlings were available. The record for the eight-leaved seedlings is so imperfect that it is omitted (Table III).



Seedlings of biennial (2066, three rows at the right) and of annual white sweet clover grown in greenhouse December 14, 1921, to February 13, 1922, under normal day (top row), short day (middle row), and long day (bottom row)



Annual and biennial white sweet clover grown under normal day in greenhouse January 12 to March 13, 1922. (Compare with Plate 1)

The measurements given in Table III bring out the same facts as those recorded in Table II. Under normal day the internodes of the annual were generally longer than those of the biennial (pl. 3), but this difference tended to disappear under longer illumination although, even in the older seedlings, the internodes of the annuals always averaged somewhat longer than those of the biennials. This is true of the first as well as the subsequent internodes (pls. 4 and 5).

In the winter of 1922-23 this work was repeated, the following lots of seed being used:

- 2062. Common biennial.
- 2066. Grundy County biennial.
- 2143. Mixed annual.
- 12044. Late annual.
- 12001. Early annual.
- 12056. Late annual.

The seeds were sown November 16, and some were measured January 10, 55 days from sowing; others January 25, 70 days from sowing. Those of the first set were nearly all two-leaved, those of the second nearly all three-leaved. Both sets of figures are given in Table IV:

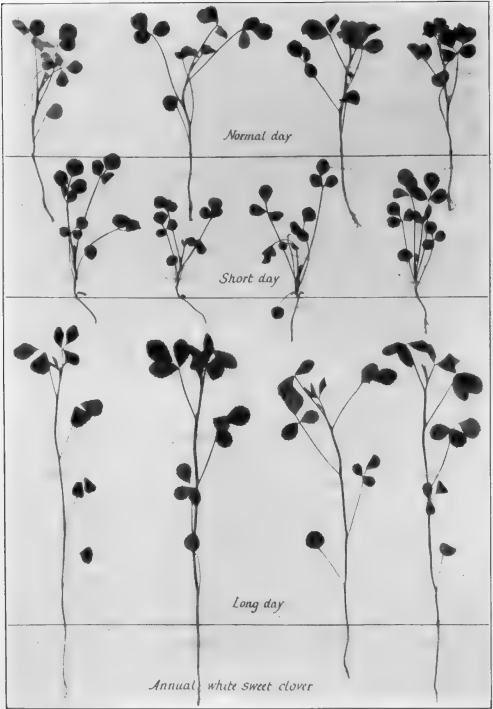
TABLE IV.—Average length of first internode, average stem length, and average internode length of two-leaved seedlings measured 55 days and of three-leaved seedlings measured 70 days after sowing on November 16, 1922

[Measurements in millimeters. Lettering as for Table II]

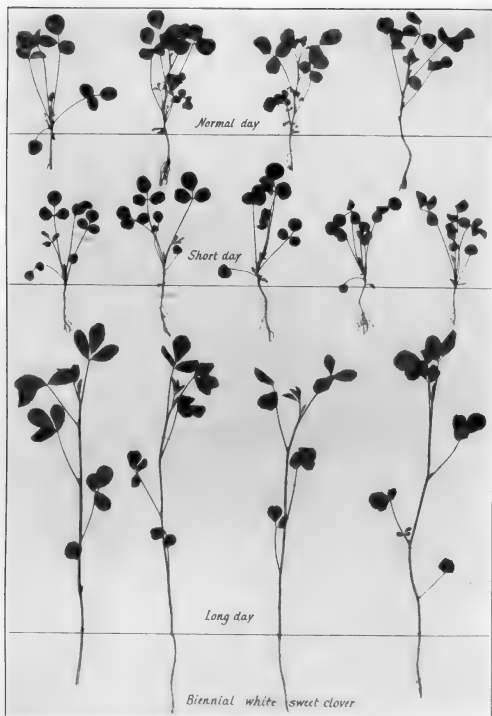
		2-leaved (55 days)						3-leaved (70 days)					
		Biennial		Annual				Biennial		Annual			
		2062	2066	2143	12044	12001	12056	2062	2066	2143	12044	12001	12056
NF	I'-----	6.2	4.7	6.6	9	5.7	4.3	5	2.3	5.8	7	5.7	5.3
	S-----	10.7	6.4	11.5	14.7	12.1	8	10.6	6	17.4	17	24.5	13.7
	I-----	2.2	.8	2.5	2.8	3.2	1.8	1.5	1.2	3.9	3.3	6.3	2.8
N	I'-----	6.6	4.3	8.6	9.4	7.1	6.8	8.4	5	7.4	9.4	8	6
	S-----	15	6.9	18.2	18.4	17.3	14	21.8	10.2	24	28.4	29	22.2
	I-----	4.2	1.3	4.8	4.5	5.1	3.6	4.5	1.7	5.5	6.3	7	5.4
S	I'-----	6.4	4.3	8	11.7	6.5	6.4	8	3	6.4	8	7	6.1
	S-----	10.6	6	15	29	14.2	12.4	17	4.7	13.4	17.4	23	15
	I-----	2.1	.8	2.5	8.6	3.8	3	3	.6	2.3	3.1	5.3	3
L	I'-----	16.5	12.3	15.5	22.4	13.4	12.1	15	11.6	15	23	14	13
	S-----	35	28	35	46.8	29.9	26	37.6	39	49.3	65	48	45
	I-----	9.2	7.8	9.8	12.2	8.3	6.9	7.5	9.1	11.4	14	11.3	10.7

All flats were in a center bench, some 6 feet below the glass, and it was suggested that if the seedlings, under normal day, were nearer the glass, the stems might be shorter. To test this, one set of flats was placed on a frame raised so as to bring the surface of the soil within 8 or 9 inches from the glass. In Table IV, the measurements of the seedlings taken from these flats are given under NF.

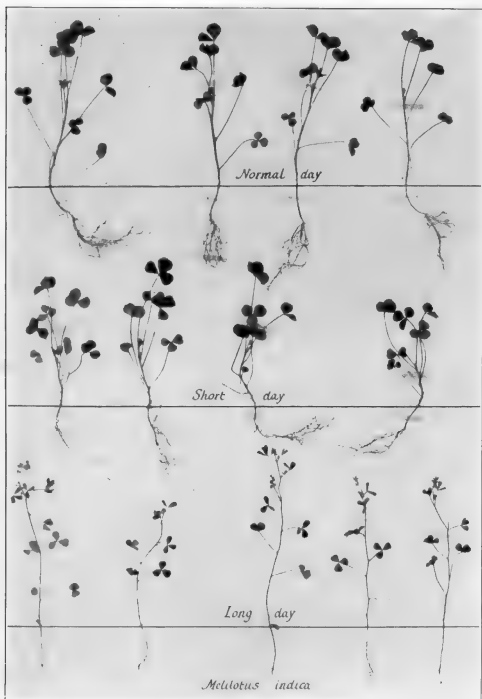
As between the seedlings, under normal day, from the center bench and those from the raised bench, the length of the first internode does not show any consistent difference in the younger seedlings, but does in the older ones. In the three-leaved seedlings the first internode of those from the raised bench is regularly and noticeably shorter than in those seedlings growing farther from the light. This feature comes out more prominently when the stem lengths of the two sets



Annual white sweet clover seedlings grown under normal, short, and long day in greenhouse January 12 to March 13, 1922, showing the effect of photoperiodicity



Biennial white sweet clover seedlings grown under normal, short, and long day in greenhouse January 12 to March 13, 1922, showing the effect of photoperiodicity



Seedlings of *Melilotus indica* grown under normal, short, and long day, November 16, 1922, to February 17, 1923, showing effect of photoperiodicity. Note the small leaves, blossoms, and young pods on plants 93 days from seeding

are considered. In both two-leaved and three-leaved seedlings the plants from the raised bench are shorter than those grown farther from the light. In many cases this length is nearly the same as that secured under the short day.

Comparing these two sets with those grown under normal day at about 6 feet from the glass, it will be noted that the effect of growing the seedlings near the glass has been expressed in the same direction and often to the same degree as has that of decreasing the time of illumination. Increasing the duration of illumination has again resulted in a marked increase in length.

One trial with *Melilotus indica* may be mentioned here as an example of the striking effect photoperiodicity may exert. Seeds of this species were sown November 16, 1922, and the seedlings removed and pressed February 17, 1923. The behavior of those under short day was not at all remarkable—indeed they can not be readily distinguished from those grown under normal day—but the seedlings under long day show striking changes (pl. 6). Not only have the internodes been lengthened, but the plants have flowered and fruited when only 93 days old and barely 9 to 11 cm. long. Moreover, the leaflets are decidedly smaller than those of seedlings grown under normal day. This behavior of *Melilotus indica* under long day is quite in harmony with its field behavior. When grown in the South, it blooms as soon as the days get longer in spring, and in the North it has no value because it runs to bloom when only a few inches high. It has been thought that the warm days of a northern May accounted for this, but it now seems probable that the long period of sunshine rather than the temperature is the exciting cause.

CONCLUSION

It has been shown that the internodes of the annual white sweet clover are longer than those of the biennial. This is most marked in seedlings grown out of doors in late summer and is then sufficient in degree to serve to distinguish the two. Under a short or a long day in the greenhouse the differences tend to disappear, and controlling the period of illumination can not be recommended as a useful method in this case, though Oakley and Westover found it useful in alfalfa varieties.



JOURNAL OF AGRICULTURAL RESEARCH

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SCLEROTINIA SPECIES CAUSING DECAY OF VEGETABLES UNDER TRANSIT AND MARKET CONDITIONS¹

By G. B. RAMSEY²

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INTRODUCTION

Sclerotinia sclerotiorum (Lib.) Masee (*Sclerotinia libertiana* Fekl.)³ has long been recognized as an important pathogene in Europe. As early as 1886 DeBary (2)⁴ made a comprehensive study of this fungus and showed that it was able to produce disease in a variety of plants. In America, apparently, no reference was made to this fungus until about 1890, when it was found associated with lettuce diseases of the type called "drop." It remained for R. E. Smith (28), however, to definitely establish, in 1900, the causal relationship of *S. libertiana* to lettuce "drop" and to clear up much of the misunderstanding regarding the life history of this fungus. Previous to this time several diseases of this type were fairly well known, but the causal organisms had not been established. This is shown by the articles of Humphrey (17), Selby (26), Stone and Smith (30), and Ramsey (25). Undoubtedly *Sclerotinia*, *Pythium*, *Botrytis*, and *Rhizoctonia* were individually and collectively responsible for most of the diseases described by these investigators.

PURPOSE OF STUDY AND SOURCE OF MATERIAL

At the present time it is found not only that *Sclerotinia libertiana* and related species are causing enormous damage in the field, but also that these fungi are the cause of some of the worst storage and transit troubles. It was with the purpose of becoming better acquainted with the latter type of decay and the causal organisms involved that the present work was undertaken.

In this study four main objects were kept in view: (1) To collect a large number of cultures from all available hosts and to make morphological and physiological studies, to determine, if possible,

¹ Received for publication Oct. 1, 1924; issued December, 1925.

Contribution from the Research Laboratory on Market Diseases of Vegetables and Fruits, Bureau of Plant Industry, United States Department of Agriculture, and the Botany Department of the University of Chicago, cooperating.

² The writer is greatly indebted to Dr. Geo. K. K. Link of the University of Chicago for valuable suggestions and for criticism of the manuscript. Thanks are also due Prof. H. H. Whetzel of Cornell University for suggestions on the studies of the microconidia and for the use of his laboratory during the month of July, 1920.

³ According to the rules of nomenclature, the name *Sclerotinia sclerotiorum* (Lib.) Masee, is to be preferred, but since the name *S. libertiana* Fekl. has been used exclusively in American literature, it seems best to carry this name in the present paper in order to avoid confusion (31).

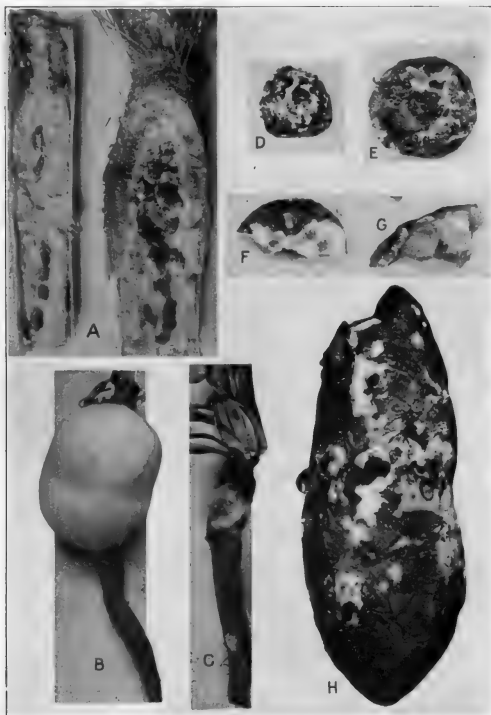
⁴ Reference is made by number (*italic*) to "Literature cited," p. 631.

whether one or more species were involved; (2) to make cross inoculations with these cultures, to see whether or not there were any specificities which cause certain strains to infect definite hosts; (3) to make temperature studies of these fungi; (4) to determine the rôle of the microconidia in the life history of the genus *Sclerotinia*.

The material and cultures used in the following studies were all collected by the writer on the Chicago market, with the exception of a few strains, for which due credit is given in the footnotes. Material from practically every State has been collected and cultured. This has offered an admirable opportunity to compare strains of the genus *Sclerotinia* from all of the important truck-crop regions, and it is believed that the studies given here will be of special value in that such a wide geographical range and large variety of hosts were considered. The literature of the subject abounds with such excellent descriptions of the field diseases caused by *Sclerotinia* that it is felt hardly worth while to do more here than cite some of the more important articles. While the bibliography at the close of this article does not pretend to be complete, it is hoped it will furnish a working basis for any who may be interested in this subject.

ECONOMIC IMPORTANCE

Among the numerous fungi that are important in causing diseases in vegetables under transit, storage, and market conditions, members of the genus *Sclerotinia* rank well toward the head of the list. This fact has been especially noticeable since the establishment of the Food Products Inspection Service by the Bureau of Agricultural Economics of the United States Department of Agriculture. More than 30,000 inspections of perishable fruit and vegetable produce are now made at the receiving markets annually, and the inspectors' certificates on car-lot shipments covering a great number of vegetables show a surprisingly large loss due to decay caused by *Sclerotinia* species. In their studies of the market diseases of vegetables, Link and Gardner (20) adopted the term "watery soft rot" to describe the decay caused by *S. libertiana*, and said: "In the market it occurs on a wide variety of hosts. It is the prevalent rot at low temperatures, and is the most important storage rot of root crops." The type of decay produced varies somewhat with the different species and the different hosts, but there are some outstanding characteristics which are usually common to all. Chief among these is the soft, watery, odorless type of decay. If the host tissue is free from other contaminating organisms, it may become so completely penetrated by *Sclerotinia* mycelium that practically all of the water will be liberated without odor. In fact, the liberated juice of the host seems to increase the natural odor of the plant. A second characteristic is the abundant white cottony mycelium which is present on the surface of the host in a humid atmosphere, and the black sclerotia which are formed in the old mycelium in regions where the host tissues are partially consumed (pl. 1, B, C, H). The sclerotia are normally produced on the surface of the host, but in some instances under field conditions they are formed within the pith of stems, such as those of the potato, tomato, sunflower, and the seed stalks of lettuce (pl. 1, A). In such cases the sclerotia are often



Mycelial and sclerotial development of *Sclerotinia libertiana* and *S. minor* on various substrata

- A.—Sclerotia of *S. libertiana* formed within the seed stalk of lettuce
 B.—Luxuriant cottony growth of mycelium of *S. libertiana* on salsify root held in a moist chamber at room temperature
 C.—Growth of *S. libertiana* on salsify under moderately dry conditions
 D, E.—Mycelial and sclerotial development of *S. minor* on slices of parsnip and carrot, respectively
 F, G.—Mycelial and sclerotial development of *S. libertiana* on slices of parsnip and carrot, respectively
 H.—Sweet potato attacked by *S. libertiana*, showing development of large sclerotia

closely crowded together and take up most of the space in the hollow or semihollow regions.

In hampers of green beans and peas it is not uncommon to find the pods covered and joined together by the white, cottony mycelium, which grows luxuriantly in the humid atmosphere in the center of the container. This "nested" condition is common in wet seasons when field conditions are favorable for infection and development of the fungus. Hampers which contain only a few affected pods at time of shipment may show a relatively high percentage of infection and decay upon arrival at market. After selecting at random 42 cars of green beans, which on inspection at market showed *Sclerotinia* decay, it was found that the percentage of infection ranged from 5 per cent in some cars to as high as 75 per cent in others, the average for all the cars being 24 per cent. Random selection of 12 cars of green peas showed an average of 20 per cent infection and decay caused by *Sclerotinia*.

All of the common root crops, such as parsnip, salsify, turnip, rutabaga, and carrot are affected in about the same manner. The soft, watery, odorless decay is produced; and if the host tissue is white, as in parsnips, salsify, and turnips, a slightly pinkish color is often quite noticeable on the margin of the lesion, while the inner portion of the lesion is pale brown and water-soaked. The pink discoloration is also sometimes evident in celery, cabbage, and cauliflower.

In Table I are listed the most important truck crops that are subject to the attacks of *Sclerotinia*, and the States which ship the bulk of these products. This table shows that the fungus has a wide host range and that it is found wherever truck crops are grown. Several hosts of minor economic importance, such as cress, escarole, mangel, and others, are not included. The tomato fruit is also omitted because of the absence of an authentic record of the occurrence of *Sclerotinia* decay of tomatoes on the market, although the tomato fruit is very susceptible to decay when inoculated with all strains and species of *Sclerotinia* which the writer had under observation. In 1918, watery soft rot was reported in two cars of Florida tomatoes by the food products inspectors, but as it has never been found since then there may be some doubt as to the correctness of the diagnosis.

Tomatoes grown under glass are more liable to infection by *Sclerotinia* than those grown in the open field. McClintock (21) reported a fruit rot due to *Sclerotinia libertiana*, and Dickson (11) has reported a wilt of tomato plants in the greenhouse, on soil previously cropped to lettuce which had suffered an attack of "drop." A blossom blight of tomatoes in Albany County, N. Y., was also reported to the Plant Disease Survey in 1920 by H. W. Fitch. The causal organism in each of these cases was apparently *S. libertiana*. In April, 1923, W. A. Orton and A. C. Foster found tomato plants near Sanford, Fla., which were affected with a sclerotia-forming fungus, and material sent to the writer showed numerous small, irregular black sclerotia in the pith regions of the stems. Cultural and cross-inoculation work indicates that this organism is identical with *S. minor*, which is reported by Jagger (18) to affect lettuce in that locality.

Table II,⁵ compiled from the reports of the food-products inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture, for 1920, presents in a concise form the damage sustained by that part of the carrot crop which was inspected in our principal markets. Practically half of the cars inspected showed more or less decay (watery soft rot) due to *Sclerotinia*. The range up to 100 per cent infection in advanced stages shows how serious the ravages of this fungus may be.

TABLE II.—*Prevalence of Sclerotinia watery soft rot of carrot as determined by food-products inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture, 1920*

Origin of shipment	Number of cars inspected	Number of cars with decay	Average per centage of decay	Date of inspection	Market where inspected	Percentage of decay	Remarks
California.....	7	3½	14	1920 Mar. 31 June 30 July 1 Aug. 9 Dec. 17 Dec. 20	St. Louis..... Chicago..... St. Paul..... New Orleans..... Boston..... Chicago..... do..... do..... do.....	12 to 15 3 to 5 20 to 30 10 to 15 4 25 to 30 75 to 100 90 80 to 90 25	One-third of car. Large pits at stem. Advanced stage. Do.
Illinois.....	4	4	52	Mar. 16 June 17	do..... Detroit.....	90 25	With Rhizopus rot, worst in upper tiers. Decay in tops.
Louisiana.....	2	2	55	June 18	Minneapolis.....	40 to 50	
Mississippi.....	1	1	45	May 6	Washington.....	5	
New York.....	20	4	10	Nov. 1 Nov. 9 Nov. 13 Dec. 9 Jan. 12 Feb. 9 May 4 May 28 Feb. 16 Mar. 17 Oct. 29 Nov. 30	New York..... do..... Pittsburgh..... do..... New Orleans..... Minneapolis..... St. Louis..... Chicago..... New York..... Pittsburgh..... do..... Washington.....	15 to 20 15 3 to 5 20 to 25 25 to 30 10 to 15 2 25 to 50 15 15 15 to 20 35 to 60	In tops. With slimy soft rot. With gray mold rot and Rhizopus rot.
Ohio.....	2	1	22	Dec. 9	do.....	20 to 25	
Oregon.....	2	2	20	Jan. 12	New Orleans.....	25 to 30	
Texas.....	3	2	20	Feb. 9	Minneapolis.....	10 to 15	
Unknown.....	11	4	24	May 4 May 28 Feb. 16 Mar. 17 Oct. 29 Nov. 30	St. Louis..... Chicago..... New York..... Pittsburgh..... do..... Washington.....	2 25 to 50 15 15 15 to 20 35 to 60	

Total number of cars inspected..... 52

Total number of cars with watery soft rot..... 23½

Similar figures for the lettuce crop of 1920 show that of 613 cars inspected, 108 had decay due to *Sclerotinia*. In 36 of these cars, between 75 and 100 per cent of the heads were infected. These heads are not usually a total loss, but any considerable amount of infection necessitates severe trimming before the product can be placed on the market. In the cars showing *Sclerotinia* decay that were shipped from the eight leading lettuce-producing States during 1920, an average of 54 per cent of the heads was affected.

Watery soft rot is also one of the most severe transit rots of celery. About half of the car-lot shipments which the Bureau of Agricultural Economics is called upon to inspect show more or less of this decay. In 1920 more than 365 cars were inspected, and of this number 207

⁵ HASKELL, R. J., and WOOD, J. I. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1920. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Surv. Bul. Sup. 16, p. 271, table 100. 1921. (Mimeographed.)

were affected with *Sclerotinia* decay. The percentage of infected plants in these cars ranged from 1 per cent in some cars to 100 per cent in others, the average being approximately 50 per cent.

Sclerotinia libertiana occurs much less frequently on fruits than on vegetables. The writer has studied a strain from lemon,⁶ which was known to cause "cottony rot," and also a culture from strawberry. Smith (27) concluded from his morphological and cross-inoculation work that the organism causing "cottony rot" of the fruits and attacking the twigs of oranges and lemons in the orchard is identical with *S. libertiana*. In the present work, the writer has been unable to find any differences between the strains just mentioned and many other strains obtained from vegetable crops. The sclerotia, apothecia, asci, and spore measurements are similar, and inoculation work has shown these strains to be pathogenic to all of the vegetables tried, except potato. It appears, therefore, that *S. libertiana* is not limited to the vegetable crops, but that the manner of growth of the host and the opportunities for infection are the important limiting factors.

A study of climatological, together with the inspectors' certificates issued by the United States Bureau of Agricultural Economics on car-lot shipments of perishable produce, show that a rainy season just before and during harvesting time is very favorable for *Sclerotinia*, and that crops are more likely to become infected under such conditions and decay during transit or storage. This is very well illustrated by a refrigerated car of Louisiana carrots inspected by the writer in Chicago. These carrots were wet and dirty and gave every evidence of having been dug in wet weather. They were inspected and accepted at loading point as a good product. The car was in transit only four days, and its refrigeration record (40° to 46° F.) was good throughout the trip, yet on arrival at Chicago careful inspection showed that more than 85 per cent of the carrots were affected with *Sclerotinia* decay. The tops were wet and badly decaying, and the roots showed watery lesions often covering over 25 per cent of their surface. This is also a very good illustration of the fact that products which appear healthy at loading time may be contaminated or may have incipient lesions which develop into serious decay in transit. There was a loss of approximately \$900 on this car due to this decay. This is only one of the many instances that might be cited to show the swift destruction of vegetable produce caused by *Sclerotinia* under refrigeration and transit conditions that are ordinarily considered good.

MORPHOLOGY

A large number of cultures have been compared in an attempt to ascertain whether there were any morphological differences between the various strains of *Sclerotinia* collected, as shown by mycelium, sclerotia, apothecia, asci, spores, and microconidia.

In culturing fungi, it has sometimes been noticed that the type of inoculum used in making the transfer determines, to some extent at least, the type of growth and spore formation (1). In transferring from old cultures of *Sclerotinia*, the sclerotia are probably most often

⁶Original culture obtained from H. S. Fawcett, through courtesy of D. H. Rose.

used as inocula, while from young cultures the mycelium usually is transferred. The writer had never noticed any difference between cultures raised from sclerotia and those grown from mycelial transfers, but in order to get definite data on this point and to note in particular whether there was any tendency to change the manner or amount of microconidial production, or a tendency for other conidial forms to develop, the following experiment was conducted. Six cultures were selected, five of *S. libertiana* and one of *S. intermedia* (24). From these cultures, two sets of transfers were made. In the first set only mycelium and the associated microconidia were used as inocula, while in the second set care was taken to transfer only sclerotia. These two sets of cultures were grown at room temperature in diffused light, and were transferred, in the manner just described, every month for nine successive months. Notes were taken on each successive generation at the end of one month's growth, in an effort to discover any differences in growth and development between the cultures from the sclerotial and those from the mycelial transfers. At the close of the period there was nothing to indicate that it would be worth while to continue the study. The results of this experiment can be summarized by saying that the chief difference noted was in the rate of growth. The mycelial transfers grew much faster immediately on being placed on new agar, consequently producing more vegetative growth and forming sclerotia sooner than the cultures arising from the sclerotial transfers. The slower-growing sclerotial plantings, however, were comparable to the other transfers by the end of the month. There was no tendency to change from the normal way of growing in either series, and no more sclerotia or microconidia were produced than usual.

EFFECT OF TEMPERATURE AND CULTURE MEDIA ON SCLEROTIA

Cultures of *Sclerotinia* grown at room temperature maintain approximately the same relative size of sclerotia for a given species, provided a suitable medium is used. In cultural studies, however, the writer has often noted that the relative size of the sclerotia of different species does not remain the same when extremes of temperature are involved. In general, when the temperature is near the minimum growing point for the fungus, the sclerotia have a tendency to be larger than the normal for room temperature. This is particularly so in the small sclerotial forms. *S. intermedia* on potato dextrose agar at 5 to 7° C. has been observed to form sclerotia 2 to 4 mm. in diameter, while the normal for this species is 2 mm. at 20°. On the other hand, sclerotia grown near the maximum for the fungus have a tendency to be smaller than normal for room temperature. Sclerotia of *S. libertiana*, which usually average about 4 mm. in diameter at 20°, have ranged from 1.5 to 4 mm., with an average diameter of 2.5 mm. when grown on the same medium at 30°. *S. intermedia* grows very poorly at this temperature and the sclerotia formed vary from 1 to 2 mm. in diameter.

Changes in the size of the sclerotia have also been noted in cultural studies when apparently other factors than temperature were involved. A *Sclerotinia* was isolated from parsnip which produced a scant mycelium with numerous very small sclerotia. The first isolation plate and the first transfer to tubes of potato-dextrose agar

gave sclerotia which would average less than 1 mm. in diameter. After several transfers on potato-dextrose agar, the sclerotia were 2 to 5 mm. in diameter, with an average diameter of about 3 mm. This leads one to suspect that the fungus had become better adapted to the artificial medium. Gilbert and Bennett (14) found in their study of *Sclerotinia trifoliorum* Erik. that large sclerotia were produced on favorable media such as potato plugs, while on unfavorable media the sclerotia were small. This undoubtedly explains why the sclerotia on the host plant are often quite different from those of the same fungus when grown on artificial media. From these observations it is quite evident that while the size of the sclerotia may be useful in separating species, this character can not be depended upon unless the influence of temperature and culture media are taken into consideration.

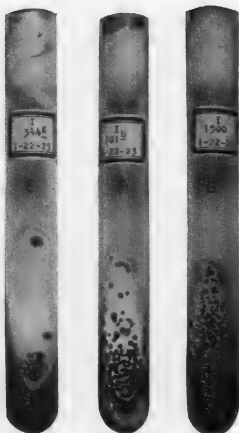
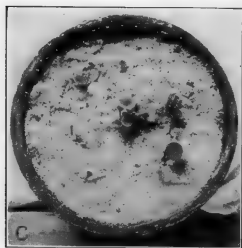
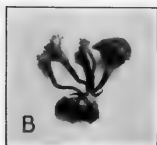
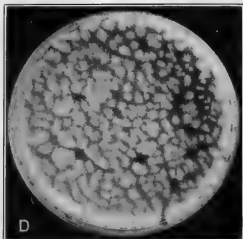
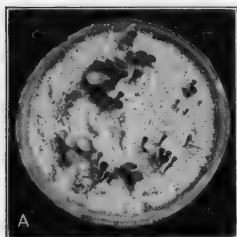
The sclerotia of *S. minor* and *S. intermedia* have showed constant differences from those of *S. libertiana* throughout all experiments. In all cultures of the large sclerotial type there were no appreciable differences in the sclerotia of the strains studied that could not be attributed to growing conditions. As it is fair to compare only sclerotia that have been grown under exactly parallel conditions, the following data will suffice to show the relative size of sclerotia of the strains of *Sclerotinia* under consideration. On plate cultures of oat-meal agar, the large sclerotial strains produced sclerotia ranging from 2.5 to 6 mm. in diameter, averaging 3.5 mm. In similar cultures of *S. minor* the range was 0.5 to 1 mm., average 0.78 mm. in diameter, while the sclerotia of *S. intermedia* ranged from 1 to 3 mm. in diameter, with an average of 2 mm. These species cultured on tubes of potato agar are shown in Plate 2, E, F, and G.

APOTHECIAL PRODUCTION

Laboratory experiments show that no definite period of dormancy is necessary to prepare sclerotia for production of apothecia. Sclerotia held in a dry state for several months do not germinate any more readily than those of the same kind which are one or two months old. No advantages have ever been observed from chilling or alternating the temperatures at which the sclerotia were held. There are, however, differences which are traceable to seasonal or ecological conditions, such as the influence of light, temperature, and moisture.

The development of apothecia may be divided into two phases. The first embraces the germination of the sclerotium and sending forth of the hornlike stalks; the second involves the development of the funnel-shaped top and its ultimate expansion into the disklike cap. Experiments show that germination of the sclerotia is dependent upon proper moisture and temperature conditions, while the main factor for the development and full expansion of the apothecium is light.

Best results in the laboratory have been obtained by planting mature sclerotia 5 to 10 mm. deep in sterile sand in an ordinary porous flower pot (pl. 2, A and C). Four-inch pots placed in saucers or halves of Petri dishes filled with sterile water have been found satisfactory if they are placed under bell jars. The use of sterile pots, sand, and water has been necessary in most instances in order to



Some developmental stages in the life history of *Sclerotinia*

- A.—Apothecia of *S. libertiana* grown in flower pots of sterile sand
 B.—Malformed apothecia of *S. libertiana*, produced under unfavorable growing conditions
 C.—Apothecia of another strain of *S. libertiana*. Compare with A
 D.—Plate of potato-dextrose agar showing numerous colonies of growth from germinating ascospores. Plate inoculated by being held over a "shooting" apothecium
 E, F, G.—Parallel cultures of *S. libertiana*, *S. intermedia*, and *S. minor*, respectively, grown on potato agar

avoid the interference of algal growth on the surface of the sand and on the edges of the pots. These pots should be placed in rather strong diffused light. Those placed in the north and west windows of the laboratory have produced apothecia abundantly during the late winter and spring months.

Although sclerotia have been planted in sand during practically every month of the year, by far the greatest number have germinated and produced normal apothecia during the months from January to April inclusive (Table III). Since the water was administered artificially in all instances, this factor may be considered constant, thus leaving the variable factors of light and temperature to be determined. No way of sharply separating the influences of light and temperature has been found. Nevertheless, since sclerotia have been germinated in the dark when they were held at 18° to 22° C. during the unfavorable summer months and whereas they did not germinate in the dark at laboratory temperatures (22° to 30°) during this time, it seems safe to assume that temperature was the more important limiting factor.

TABLE III.—*Germination of sclerotia of Sclerotinia species in sand at laboratory temperature*

SCLEROTINIA LIBERTIANA

Source of culture	Date of culture	Date of planting sclerotia	Date of germination	Apothecia			
				Number started	Number matured	Diameter	Height above sand
						<i>Mm.</i>	<i>Mm.</i>
Bean.....	Jan. 4, 1922	Oct. 5, 1922	Feb. 2, 1923	6	6	3	3 to 6
Carrot.....	Aug. 9, 1921	Mar. 9, 1922	Apr. 4, 1922	3	3	1 to 3	4 to 8
Do.....	July 14, 1921	do.....	Apr. 10, 1922	3	3	3 to 5	4 to 6
Cauliflower.....	July 1, 1922	Oct. 6, 1922	Feb. 10, 1923	1	1	7	10
Celery.....	Aug. 1, 1921	Jan. 30, 1922	Feb. 20, 1922	15	10	2 to 6	5 to 12
Chicory.....	Jan. 25, 1922	Oct. 6, 1922	Mar. 15, 1923	4	1	2	4
Endive.....	July 28, 1921	Oct. 7, 1922	Nov. 14, 1922	9	2	4	5
Lemon.....	Oct. 10, 1922	Nov. 14, 1922	Jan. 30, 1923	15	5	4 to 6	4 to 8
Lettuce.....	June 15, 1921	Oct. 5, 1922	Feb. 21, 1923	8	7	3 to 6	5
Do.....	Direct from host.	Feb. 2, 1923	Mar. 5, 1923	69	8	5 to 13	4 to 10
Do.....	June 2, 1921	Jan. 30, 1922	Feb. 17, 1922	6	3	4 to 5	4 to 6
Parsnip.....	July 28, 1921	Mar. 9, 1922	Apr. 15, 1922	14	12	4 to 7	6 to 8
Salsify.....	do.....	do.....	Apr. 22, 1922	6	6	3 to 6	5 to 8
Strawberry.....	Oct. 8, 1920	Feb. 24, 1921	May 12, 1921	3	1	4	5
Sweet potato.....	Apr. 1, 1922	Nov. 8, 1922	Jan. 9, 1923	16	3	3 to 4	5 to 7
Do.....	Direct from host.	Dec. 28, 1922	Feb. 7, 1923	88	14	3 to 5	3 to 6
Turnip.....	June 12, 1921	Jan. 30, 1922	Feb. 20, 1922	3	3	5	5 to 8

SCLEROTINIA INTERMEDIA

Salsify.....	Sept. 14, 1920	Apr. 10, 1921	Apr. 28, 1921	18	10	2 to 4	5 to 8
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Continued warm weather seems detrimental to apothecial production, but apothecia will form when the temperatures alternate between night and day, providing the extremes are not too great. This is shown by the development of normal apothecia in the winter months of December, January, and February, when the day temperature ranged between 20° and 22° C. and the night temperature between 13° and 18°.

Experiments do not show that any definite time is necessary between the planting of sclerotia and their germination. Sclerotia planted in

August have germinated in January, and sclerotia planted in April have germinated and produced apothecia in April. The shortest time between planting and germination was 18 days, while the longest was 141 days. From this it seems safe to assume that the time of apothecial production depends entirely upon ecological conditions such as moisture, temperature, and light. Variations in the length of time between date of planting and date of germination, as shown in Table III, must be attributed to these ecological factors rather than to differences between strains of *S. libertiana*.

Small sclerotia are especially susceptible to decay when the pots are kept too moist, and for this reason it is usually much more difficult to obtain apothecia of the small sclerotial forms than from those of the *S. libertiana* type. Various investigators have found that the sclerotia of *S. libertiana* will remain alive in dry state for several years, but it is usually agreed that in nature most of them decay during the first year. In this connection it is interesting to note, however, that Pollock (23) has given evidence to show that the sclerotia of *Sclerotinia cinerea* (Bon.) Schrot. may remain alive for 10 years in fallen mummified fruits.

As noted by Stevens and Hall (29), the light factor has been found to be especially prominent in stimulating the full development and expansion of the disk-shaped apothecium. Small, hornlike outgrowths are often found growing from sclerotia in old test-tube cultures which have been stored in the dark, but the writer has never known of one to expand into a normal apothecium. These aborted apothecia are quite common in sand-pot cultures that have been held in weak light. In some experimental pots used by the writer, more than 100 apothecial stalks have started out normally and then failed to expand presumably because of unfavorable light conditions. When these pots were placed in good light, the apothecia which started out subsequently developed in a normal manner. Abnormal and freakish apothecia are often formed when unfavorable growing conditions alternate with conditions favorable to growth. This stopping and resuming of development often leads to such freaks of apothecia as shown in Plate 2, B.

SINGLE ASCOSPORE ISOLATIONS

When apothecia are allowed to mature and fully expand under a bell jar, it is comparatively easy to obtain single spore cultures. A smokelike cloud of ascospores is discharged almost immediately upon a change of humidity brought about by lifting the bell jar. This cloud of ascospores is visible even when only one apothecium discharges, owing to the fact that even a moderate-sized apothecial cup may contain an enormous number of spores. Stevens and Hall (29) estimated that one cup may produce 31,000,000 spores. If a large number of apothecia are ripened at the same time and held under cover so that their discharge can be controlled, it has been found possible to photograph this cloud of spores as it rises into the air.

Dickson and Fisher (12) devised a method by which an excellent photograph of the spore-cloud emanating from apothecia of *S. libertiana* was obtained. By holding a Petri dish containing a thin layer of nutrient agar inverted over a discharging apothecium, a large number of the spores are shot against agar surface. The

spores thus caught on the agar film can be seen with the low power of the microscope and marked by a dot of ink on the bottom of the Petri dish. By choosing a thinly seeded portion of the plate for marking the spores, and by using reasonable care, a small portion of agar above the marked location of the spore can be lifted out without fear of getting more than one spore. If, however, the agar is cloudy and there is doubt as to the location of the spores, it has been found practical to hold the Petri dish culture for 12 hours, or until short germ tubes have started. When observations are made early it is not difficult to locate single germinating ascospores.

ASCI AND SPORES

The spore and ascus measurements are very important in distinguishing between species of the genus *Sclerotinia*, and for that reason a large number of measurements from numerous cultures have been made. The average measurements of typical large sclerotial strains of *Sclerotinia* from important host plants are listed in Table IV. For the sake of comparison the corresponding measurements of *S. minor* (18), *S. ricini* (15 and 16), and *S. intermedia* (24) are also given. This table shows a striking similarity in spores and asci of the 28 strains belonging to the large sclerotial type herein listed. While there is a slight variation between strains from different hosts, this difference is no greater than that within strains from the same host or the variations even within a given strain. The average measurements of asci ranged from 8.2 to 10.2 microns in width and from 125.4 to 160.4 microns in length, the mean of the averages of all large sclerotial strains being 9.2 by 144.3 microns. The spore averages ranged from 5.9 to 7.3 microns in width, and from 11.7 to 15.1 microns in length, their mean being 6.6 by 13.3 microns.

TABLE IV.—Average measurements in microns of spores and asci of several strains of *Sclerotinia libertiana* as compared with each other and with *S. intermedia*, *S. minor*, and *S. ricini*

S. LIBERTIANA

Culture No.	Host	Asci	Spores
822	Bean.....	9.5×156.4	6.7×12.7
283	Carrot.....	8.2×144.7	7.0×14.1
763ado.....	9.2×144.4	6.7×13.8
760	Cauliflower.....	8.6×142.8	6.6×13.2
215ado.....	9.9×155.0	7.0×14.0
187b	Celery.....	9.4×146.3	6.8×13.4
695do.....	9.3×143.6	7.3×12.4
S/24do.....	9.5×144.1	6.8×13.3
738do.....	9.5×151.3	6.9×13.3
345	Chicory.....	9.0×133.0	6.0×11.9
749	Endive.....	9.8×145.9	6.9×13.3
612	Lemon.....	9.4×144.6	6.6×13.1
344	Lettuce.....	8.8×130.4	6.6×13.2
693do.....	8.2×138.1	5.9×12.6
705do.....	8.6×138.3	6.2×12.8
415do.....	9.1×143.2	6.4×13.0
719do.....	9.2×155.7	6.8×13.5
1466do.....	10.2×158.5	7.0×14.0
349do.....	9.9×150.8	7.1×13.6
261	Parsnip.....	8.7×138.3	6.8×12.9
744do.....	9.0×136.9	6.5×13.9
732	Salsify.....	9.4×160.4	7.0×14.4
819do.....	8.6×141.8	6.7×14.0
820do.....	8.6×125.4	6.2×13.2
833do.....	9.6×139.8	6.9×11.7
834do.....	9.3×145.1	6.2×15.1
177	Sweet potato.....	9.0×142.8	6.4×13.0
1567do.....	9.8×152.7	6.4×12.0

TABLE IV.—Average measurements in microns of spores and asci of several strains of *Sclerotinia libertiana* as compared with each other and with *S. intermedia*, *S. minor*, and *S. ricini*—Continued

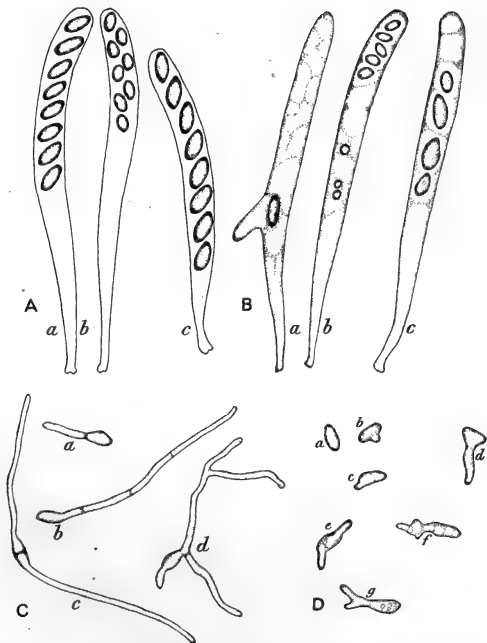
S. INTERMEDIA			
Culture No.	Host	Asci	Spores
303	Salsify.....	7.5×127.0	4.9×12.7
S. RICINI			
697	Ricinus.....	8.0×80-100	4.5×9-12
S. MINOR			
1500	Lettuce.....	8.9×141.0	7.0×14.1

Certain minor morphological variations of asci and spores are shown in Plate 3, A, *a*, *b*, *c*. These asci are each from a different strain of *S. libertiana* and they represent rather wide variations in size as well as in shape; but as more spores and asci were measured and studied the writer became more and more skeptical as to the importance of these variations. The stage of maturity of the apothecia and their variations in development due to the reactions to moisture, temperature, and light conditions are responsible for many changes in form and size of normal spores and asci, even of the same strain, as well as such unusual forms as shown in Plate 3, B, *a*, *b*, *c*, which came from the malformed apothecium shown in Plate 2, B.

The apothecial measurements of several large sclerotial strains of *Sclerotinia* and of one strain of *S. intermedia* are included in Table III. The mature apothecia that were developed in good light usually attained a diameter of about 5 mm., but in exceptional cases some grew 10 to 13 mm. in diameter. The apothecia of *S. intermedia*, for the most part, range from 2 to 4 mm. in diameter. The average height of the apothecia above the surface of the sand in pot cultures was between 6 and 7 mm. Differences in depth at which the sclerotia were planted as well as fluctuations in light, are responsible for so much variation in these dimensions that they can be considered only as indicative of the measurements that may ordinarily be expected.

MICROCONIDIA

Several groups of fungi, during some phase of their life history, develop sporelike bodies which on a morphological basis, might be called spores (pl. 4, H). Lack of definite knowledge concerning their functions, however, has caused considerable uncertainty as to the terminology to be used in speaking of these morphological units. In many ascomycetes these bodies are formed copiously by either intercalary or acrogenous abjunction and are called gonidia. They are often associated with ascocarps or other fruiting bodies and have been referred to as rudimentary spores. The spermatia of other fungi also seem to differ little if any from many acrogenously formed

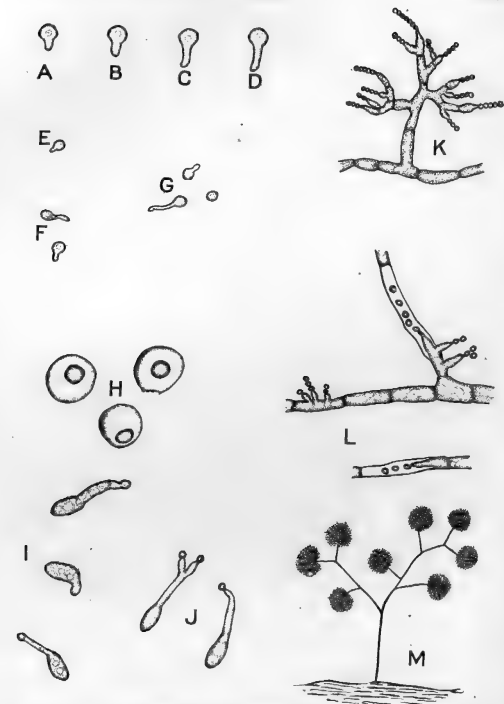
Asci and spores of *S. libertiana*

A, a, b, and c.—Showing variations in form and size of the asci and spores in different strains of *S. libertiana*

B, a, b, and c.—Malformed asci and spores from deformed apothecia similar to those figured in Plate 2, B

C, a, b, c, d.—Ascospores of *S. libertiana* germinated in distilled water at 20° C.

D, a to g.—Ascospores of *S. libertiana* germinated in distilled water at 3 to 4° C.



Stages in the formation and germination of microconidia. All drawings were made with the aid of a camera lucida

A, B, C, D.—Stages in the germination of a single microconidium of *S. libertiana* as observed under the microscope, in a hanging drop of sterile distilled water, at room temperature (20° to 24° C.). Drawings made at 3, 4, and 7 p. m. (8/10/20) and at 8 a. m. (8/11/20), respectively. No further development was made after this date

E, F.—Germinating microconidia of two other strains of *S. libertiana*, in hanging drops of water distilled in glass

G.—Germinating microconidia of *S. intermedia* in a 5 per cent sugar solution at 20° C.

H.—Microconidia of *S. intermedia* highly magnified under oil

I.—Ascospores within an overripe apothecium germinating and forming microconidia on the tips of the germ tubes

J.—Microconidia of *S. libertiana* formed on the tips of the germ tubes of germinating ascospores in distilled water

K.—Chainlike formation of microconidia upon the lateral branches of vegetative hyphae

L.—Endogenous formation of microconidia in turnip-broth cultures of *S. libertiana*

M.—Diagrammatic sketch of the botrytislike formation of microconidia of *S. trifoliorum*, as seen growing on agar plate, under the binocular

spores. In fact, the only difference seems to be in the germinating power. The bodies which germinate are called spores and those that do not germinate, or have never been observed to germinate, are called spermatia, or gonidia.

In 1918 Brierley (8) was successful in germinating the small gonidia or microconidia of *Botrytis cinerea*. He found that these microconidia germinated in form 24 to 48 hours at 17° to 23° C. when placed in water or a nutrient medium. In water the growth soon stopped, but in nutrient media it continued until a normal *Botrytis* growth was obtained. It was concluded, therefore, that the microconidia were a normal developmental stage in the life history of *Botrytis cinerea*. After studying the conditions governing microconidial formation, Brierley considered the age of the fungus to be the chief determining factor. With few exceptions, the microconidia were not found in cultures less than 30 days old. The amount of available food seemed to be next in importance, since it influenced the quantity but not the kind of spore production. Light, moisture, and temperature apparently had no particular influence upon this type of spore formation.

The microconidia of the genus *Sclerotinia* are produced in various ways. Short, lateral branches arising from the vegetative mycelium or tips of the normal hyphae may produce these sporelike bodies in great abundance (pl. 4, K, L, and M). Apparently *Sclerotinia* can produce microconidia at all stages in its life history when proper conditions are furnished. They have been found on the surface of sclerotia, on the disks of overmature apothecia which were held in a humid chamber, and on the tips of germ tubes where ascospores have germinated within the apothecium and in distilled water cultures (pl. 4, I and J).

Microconidia which are of endogenous formation have also been noted in cultures grown in rutabaga broth (pl. 4, L). Sporogenous hyphae form within some of the older vegetative cells and microconidia are cut off from the tip of the flask-shaped sterigmata which terminate these young hyphae. Vegetative cells have been found which contained large numbers of these small endoconidia. The manner of spore formation just described is practically identical with that figured and described by Beauverie and Guilliermond (4, pp. 281, 315) for *Botrytis cinerea* when grown in sterile distilled water.

The microconidia of the genus *Sclerotinia* are very similar to those of *Botrytis*, and are produced in extremely large numbers on media which are unsuitable for the vegetative growth of the fungus. Under such conditions, very scant mycelium is produced, and these small sporelike bodies are formed within a few days. On cultures of standard nutrient media, such as potato, bean, or oatmeal agar, most strains of *Sclerotinia* do not begin microconidial production until they are 20 to 30 days old.

So far as observed, the amount of available food seems to be the chief factor in determining the time of microconidial production. Abundant food of a suitable kind stimulates vegetative growth greatly, and the microconidia are not produced until the fungus has apparently used up all or most of the available surface food. As noted above, this stage is reached in most test-tube cultures about the twentieth day. Microconidia are seldom found on the natural

host plants. This is chiefly due to the fact that they are not formed until the host tissue is almost totally consumed, and when plants reach this condition they are usually thrown away without examination.

More than 200 cultures have been under observation for 3 years, and all have at some time produced microconidia in greater or less abundance. Some strains normally produced more than others, but this occurred on the general stock media, potato-dextrose agar, which was manifestly less suitable for vegetative growth of some cultures than others.

The method and time of production of these small microconidia leads one to suspect that they either function now as true spores in the life history of the fungus or that they have done so in the past. Working on this latter theory, the writer studied a large number of cultures from a wide geographical range and a large number of hosts, in the hope that some cultures would be in such a stage of adaptation or evolution that their microconidia would still be functioning. In all cultures collected the size, shape, and color of the microconidia, and the time and manner of sporulation, were essentially the same. No peculiarities or distinctions of any kind could be noted that would indicate that one culture was more likely to be viable than another.

While potato agar plus 2 per cent dextrose is a good culture medium for most *Sclerotinia* species, the addition of the dextrose seems to have a decidedly inhibiting influence on microconidial production. Few if any microconidia are formed in potato-dextrose agar cultures 20 to 30 days old, whereas on potato agar they are formed in great abundance at this age. On 20 per cent dextrose agar, a moderate growth of mycelium and a few sclerotia are produced, but no microconidia are found even in cultures 30 days old. In contrast with these results, cultures on plain potato agar and on Pfeffer's agar, held at room temperature, have formed some microconidia within 10 days, and plates of pure gelatin inoculated with *S. libertiana* have developed in 10 days a very scant, loosely appressed mycelium which bore prodigious numbers of microconidia on the surface as well as on the submerged mycelium.

GERMINATION STUDIES

While the presence of microconidia in *Sclerotinia* cultures has been noted by many writers, no one, as far as the writer is aware, has ever seen them germinate. The fact that they are borne late in culture, and when found look like a contamination, may account for lack of work on this particular phase of the life history. During the past three years the writer has devoted a great deal of time and study to the attempt to determine the rôle of the microconidia in the life history of the genus *Sclerotinia*.

The apparent *de novo* origin and rapid spread of watery soft rot in transit in such crops as lettuce, celery, cabbage, carrots, etc., has often suggested that some spore form was responsible for the primary field infection and for spreading the fungus from one plant to another during the transit period. A large part of this apparent spreading is undoubtedly due to ascospore infection from the field, but since even a small lesion in advanced stages of decay may bear innumerable

microconidia on the vegetative mycelium, it seemed of first importance to determine whether these microconidia were capable of germination. In this study the methods employed were, in general, very similar to those used by Duggar (13) in his studies on the germination of certain fungous spores. The extremely small size of the microconidia and their lack of color make working with them very difficult. In order to use the high power of the microscope, it was necessary to make practically all study with hanging-drop cultures. While miscellaneous experiments were conducted with numerous cultures from time to time in attempts to get the microconidia to germinate, the greater part of the work was centered about 50 strains of *Sclerotinia*, including *S. libertiana*, *S. intermedia*, *S. ricini*, and *S. trifoliorum*. These cultures represented a host range of 30 plants which are commonly affected by *Sclerotinia* and which were obtained from widely separated parts of the United States.

The more important nutrient solutions and materials used in the germination studies, testing out the ability of the microconidia of *Sclerotinia* species to germinate, were:

Distilled water, water distilled in glass, tap water, sterile tap water, fresh rain water, sterile rain water, liquid exudate from newly forming sclerotia, freshly cut slices of carrot roots, leaves of head lettuce, sterile distilled water with bits of fresh carrot tissue, celery tissue, and lettuce tissue added separately, freshly expressed juice of salsify roots, carrot roots, beet roots, turnip roots, rutabaga roots, lettuce leaves, celery stalks, ripe tomato fruit, lemon fruit, alfalfa stems, clover stems, decoctions of each of all these plants, soil decoction, ant decoction, beef bouillon, prune juice, sugar solutions, standard nutrient-salt solution, Pfeffer's nutrient solution, liquid gelatin, hard gelatin, potato agar, potato-dextrose agar, prune agar, and oat-meal agar.

In practically all experiments, hanging drops of the nutrient solution containing microconidia were made in Van Tieghem cells, and held at room temperature in diffused light. In most instances duplicates were placed in an incubator at a temperature of approximately 8° C. In many cases microconidia were separated from a suspension of mycelium and spores by filtering through filter paper. The filtrate containing the microconidia was then used to flood over agar and gelatin plates and to spray on host plants and on parts of their organs. As a rule, cultures were held for one week and examined daily for germination.

In the first hanging-drop cultures, tap water, distilled water, and rain water were used as a medium in which to test out the germination of microconidia. But such poor results were obtained (pl. 4, A, B, C, and D) that in order to find out whether substances present in the tap water or traces of copper in the distilled water were acting as toxic agents these were distilled in glass and used as media in further studies. These results, however, were no more favorable than the others. Short germ tubes started in several cultures within 12 to 24 hours, but the growth then stopped and no changes in temperature or light conditions were found that would induce further development (pl. 4, E and F).

From the results obtained with the water-culture experiments it appeared that microconidia belong to that class of spores which require a nutrient or some stimulating agent in order to germinate well. In view of the fact that Brown (10) found a decided stimulating effect produced by the volatile substances arising from bruised host

tissues in spore-germination studies of *Botrytis cinerea*, several experiments were devised in order to test similar effects on microconidia. To be sure that there were no small bits of mycelium in the spore suspension, the microconidia were filtered through filter paper. The filtrate, free from mycelium, was then used to inoculate freshly cut slices of carrot root and to be sprayed on leaves of head lettuce. The inoculated plants were held in moist chambers, at room temperature, in diffused light, and examined daily for symptoms of infection. Parallel with these inoculation experiments, several hanging-drop cultures were made in van Tieghem cells by adding bits of carrot-root tissue to the drops so that growth might be observed with the microscope.

The results of these experiments and others of similar nature were disappointing. No infection of the host occurred, and only an occasional short germ tube could be found in the hanging-drop cultures. Many hanging-drop cultures of microconidia in various dilutions of the freshly expressed juices of the host plants listed in a previous paragraph were made from time to time. In most of these cultures germination either was very feeble or there was none whatever. When germination took place, the germ tubes developed 2 or 3 spore diameters (7 to 12 microns) in length within 48 hours, and then stopped growth. Cooked broth or decoctions in general gave a higher percentage of germination than fresh juices of the same plants, but in none could development be induced when single spore cultures were attempted. Consequently, no culture has ever been obtained by the writer which can definitely be traced to a single germinating microconidium.

In addition, sugar solutions of various strengths, as well as the standard nutrient salt solution as used by Duggar (13), and Pfeffer's nutrient solution, were used as media. In these, as in previous experiments, only a small percentage of the spores produced germ-tubes (pl. 4, G). These ceased growing after 48 hours, and no changes in light or temperature conditions were found that would induce them to further activity.

The only cultures obtained from microconidia during these studies were from plantings made on potato and prune agar plates. Microconidia suspended in sterile distilled water were filtered through filter paper, which was found to remove particles of mycelium, and drop plantings of the filtrate were then made on hard agar in Petri dishes. No growth was visible to the unaided eye for two days, but on the third day mycelial development was observed in all plantings. Within one week these cultures made a normal growth of mycelium and sclerotia which appeared similar in every way to the usual cultures of *S. libertiana*.

It appears, therefore, that conditions must have been favorable in these experiments for the full development of the microconidia, although, as far as the writer is aware, these conditions were no different than those under which many other unsuccessful germination tests were made.

In order to test the microconidia for a reaction to oxygen, a series of hanging-drop cultures was made in the usual way. A drop of distilled water with dioxygen added as a source of oxygen was used as a medium. Cultures were made using the following percentages

of normal dioxygen (3.75): 0.5, 1, 2, and 5 per cent. The microconidia were taken from a culture 19 days old. Control hanging drops of microconidia in sterile distilled water were run parallel with the above experiment. Daily microscopic examination was made of all cultures for two weeks, but no germination was obtained. The controls as well as all other cultures remained inactive. It appears, therefore, that lack of oxygen probably is not the cause of poor germination.

So far as the writer has been able to ascertain, temperature and light conditions are not the limiting factors, either in the formation or germination of microconidia. To test out the age factor, germination studies have been carried on from cultures which varied in age from 4 days up to 157 days. The microconidia which gave most favorable germination were usually from cultures 20 to 30 days old, although apparent success was obtained from cultures as young as 7 and as old as 132 days. Because of the difficulty of definitely determining the time when the microconidia are formed, it is impossible to determine at just what age they are most likely to germinate. All observations along this line indicate that the microconidia are mature as soon as they are formed. No change in color, size, or shape is noticeable as the cultures age.

In all the experiments devised to test the germination of microconidia, approximately 10 per cent were successful. From these germinated microconidia, only two cultures ever developed a vigorous vegetative growth that could be recovered. These cultures were obtained from agar plates, and the greater percentage of other successful studies were obtained from artificial or synthetic media. In view of this and the fact that no infection of host plants or their fresh tissues was obtained, it seems highly probable that the microconidia of the genus *Sclerotinia* do not play an important rôle in the life history of this group of fungi and do not cause infection in the field, transit, or storage.

INFECTION STUDIES

Artificial infection of a suitable host is readily obtained, under favorable conditions, by inoculation with sclerotia, or vigorously growing mycelium (pl. 1, D, E, F, and G). The writer's experiments clearly indicate that the limiting factors in obtaining infection are moisture and temperature. More inoculation experiments have failed because of lack of water during the first penetration of the host tissues than from all other causes.

Wounds are not necessary for infection, but undoubtedly wounded surfaces with their exuding cell contents offering readily available food greatly facilitates the progress of the fungus. The succulent plants and plant parts are penetrated most rapidly, while the woody tissues are much more resistant. This indicates that the mechanical make-up of the plant tissues plays a part in resistance to *Sclerotinia* infection. Boyle (?) came to the conclusion that *S. libertiana* effected rupture of the host tissues solely by mechanical pressure. He found that the mycelium and appressoria of *S. libertiana* growing in turnip juice were surrounded by a mucilaginous sheath which fixed and held the "infection hyphae" to the cuticle of the host. He also noticed that the hyphal tips in contact with resistant material showed different staining from normal and that the infection hyphae

arose from these tips. Similar observations have been made on other fungi. Brown (9) found that *Botrytis cinerea* did not produce a substance in the germ tube which was capable of dissolving cuticle. Blackman and Welsford (6) found later that in the early stages of infection, *B. cinerea* effected its entrance into bean leaves solely by mechanical pressure exerted by the germ tube. It should be noted in this connection, however, that although first penetration of the cuticle is made by mechanical pressure, Boyle (7) found that as soon as the infection hypha gets within the cellular tissues, a chemical action takes place and cells die some distance beyond the invading hyphae, thus suggesting the secretion of an enzyme.

INFECTION BY MEANS OF ASCOSPORES

It has heretofore been the general belief that it was necessary for ascospores of the genus *Sclerotinia* to fall upon some dead organic matter and first establish themselves saprophytically in order to gain enough vigor to attack living plants. The work of Stevens and Hall (29) on *S. libertiana* and that of Beach (3) on *S. minor* indicated that direct infection by ascospores seldom, if ever, occurred. Pethybridge, in his investigations of the stalk or sclerotium rot of potatoes produced by *S. sclerotiorum*, writes (22):

Although these spores or, perhaps, more correctly speaking, their germ tubes, are incapable of entering normally vigorous green potato foliage and stalks, they are evidently capable of causing infection of the foliage when it is yellowing and ripening off, and probably are also capable of infecting the stalks at the scars remaining when the older leaves have fallen. During the past season at Clifden circumstances were very favourable for observations on the mode of attack of this fungus, and in very numerous instances it was abundantly clear that infection occurred first on one of the older yellowing leaves from which the fungus passed directly to the stalk which bore it.

The writer has been able to show in laboratory experiments that freshly cut slices of turnip, carrot, and lettuce leaves held over "shooting" apothecia will become so severely infected that the whole surface of the host will be covered with white, cottony mycelium, and the tissues reduced to a watery mass within three to five days. The rate of growth is comparable with that produced by a direct transfer of a vigorous mycelium from plate cultures. Successful inoculation has also been obtained by spraying an ascospore suspension in distilled water upon individual leaves of head lettuce. In this experiment, however, the growth of the mycelium was so general over the surface of the leaves that it was impossible to locate the original points of infection. These experiments show that ascospores can at least infect freshly-wounded tissues if not living cells.

CROSS INOCULATIONS

As just pointed out, a study of the cultural characteristics and morphology of the various *Sclerotinia* cultures obtained from market produce indicated that the same strain of *Sclerotinia* attacks a wide range of hosts. Cross-inoculation tests were made to determine whether there were any physiological races or strains. In general, the experiments were made at room temperature, in diffused light. Fresh host or host tissues were obtained from the market and sterilized in mercuric chloride (1 to 1,000) for a few minutes, and then rinsed in sterile water. Inoculations were made in wounds and on unwounded

surfaces, and the specimens were held in large sterile moist chambers. Vigorously growing mycelium a week old on a small piece of potato-dextrose agar was used as inoculum. The results of some of the more important experiments are summarized in Table V.

TABLE V.—Summary of cross-inoculation studies with *Sclerotinia* spp.

S. LIBERTIANA

Source of culture	Asparagus shoots	Bean pods	Beet roots	Carrot root	Celery stalks	Cucumber fruits	Lemon fruits	Lettuce heads	Parsnip roots	Pea pods	Potato tubers	Sweet potato roots	Turnip roots	Tomato fruits
Bean pods.....	+	+	(?)	+	+	+	+	+	+	+	+	+	+	+
Carrot roots.....	+	+	(?)	+	+	+	+	+	+	+	+	+	+	+
Celery stalks.....	+	+	(?)	+	+	+	+	+	+	+	+	+	+	+
Lemon fruit.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lettuce heads.....	+	+	(?)	+	+	+	+	+	+	+	+	+	+	+
Parsnip roots.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pea pods.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Potato stem.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salsify roots.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Strawberry fruit.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sweet potato roots.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turnip roots.....	+	+	(?)	+	+	+	+	+	+	+	+	+	+	+

S. INTERMEDIA

Salsify root.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-------------------	---	---	---	---	---	---	---	---	---	---	---	---	---	---

S. MINOR

Lettuce heads.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sunflower plant.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S. RICINI

Ricinus plants.....	+	+	(?)	+	(?)	+	+	+	+	+	+	+	+	+
---------------------	---	---	-----	---	-----	---	---	---	---	---	---	---	---	---

•+=result positive.

b—=result negative.

Twelve strains of the *S. libertiana* type which were isolated from different hosts were used, together with one strain each of *S. intermedia* and *S. ricini* and two strains of *S. minor*. These cultures were used to inoculate nine different hosts that are commonly affected with Sclerotinia decay. In addition, potato tubers, beet roots, and tomato and lemon fruits were inoculated, because the two former commodities do not seem ever to have been found on the market affected with Sclerotinia decay, while the two latter fruits were selected because of their relatively high acid content.

As shown in Table V, practically all of the strains obtained from the various host plants are pathogenic to those hosts commonly found attacked by Sclerotinia. Failure to get infection of the Irish potato was consistent. Both young and old tubers of several different varieties have been inoculated, but no tuber has ever shown even the slightest decay that could be ascribed to Sclerotinia infection. A culture isolated from potato plants in the field also proved negative to the tubers. These results are contrary to those obtained by Bisby (5), who inoculated potato tubers with a strain of Sclero-

tinia found parasitizing sunflowers in Manitoba and obtained positive results. The results obtained in the present experiments, nevertheless, indicate that the potato tuber is highly resistant to infection by *Sclerotinia*. This is in entire agreement with experiences in the market. Up to the present time not a single tuber has been found affected with *Sclerotinia* rot.

A stalk rot and wilt of potato plants caused by *Sclerotinia sclerotiorum* has been studied and described in Europe for several years. Pethybridge (22) observes that although the plants may be seriously affected in certain seasons, the tubers are not decayed. He made mention of the supposition that the fungus could not grow at all on potato tubers, but in 1909 and 1910 he obtained growth and development of sclerotia on slices of living tubers when he inoculated them with a pure culture of *S. sclerotiorum* obtained from ascospores.

In America, so far, the *Sclerotinia* disease of potato plants is of minor importance. The writer has worked with only two cultures that were isolated from potato plants.⁷ One of these was isolated from Florida plants, and the other from potatoes affected with a stalk decay in New Brunswick, Canada. Both of these cultures appear to be *S. libertiana*. In 1921, Lachaine (19) found 10 per cent of the potatoes in a field in New Brunswick affected with a stem rot and wilt. The causal organism was found to be a sclerotia-forming fungus which he was inclined to call *S. libertiana*. The crustlike formation of sclerotia on carrot, and the small sclerotia formed on potato agar as shown in his photographs, however, are very similar to those produced by *S. minor*.

The inoculation of beets was very largely unsuccessful or doubtfully positive, in many cases. No marked amount of decay was obtained with any strain, but in some cases the brownish discoloration and decay entered deep enough so that the fungus could be reisolated from the inner tissues. When the reisolations were successful the inoculation is marked positive in the table, but when only a slight discoloration and surface growth of the fungus was evident the experiment is marked with a question mark.

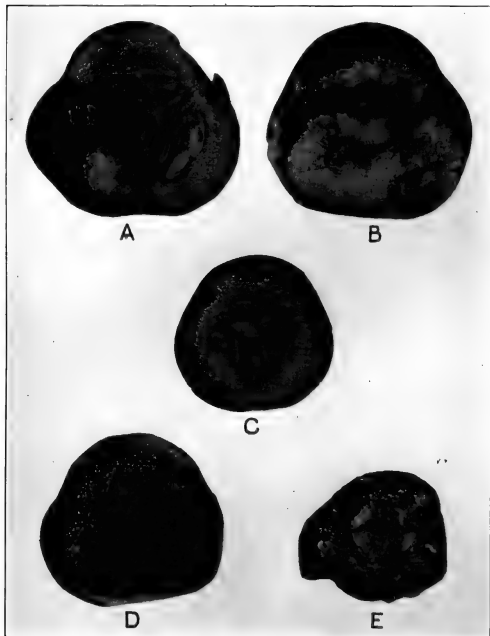
It is readily apparent, in so far as the cross-inoculation studies herein listed are concerned, that the host range of *Sclerotinia* spp. is so wide that it is impossible to separate the different species on the basis of host infection. On the hosts tried—*S. libertiana*, *S. minor*,⁸ *S. ricini*,⁹ and *S. intermedia*—all produced definite infection and decay, as shown in Table V. On most host plants the type of decay produced by the above species is quite similar and it is not until peculiarities of mycelial growth become evident or sclerotial formation takes place that the species can be distinguished (pl. 5 A, B, C, D, and E).

While there were slight variations from time to time between the growth and appearance of the *S. libertiana* strains obtained from different hosts, there were no more differences than those occasioned by fluctuations in temperature and humidity when the same strain was used in separate experiments. All observations and experiments conducted thus far indicate that all of the large sclerotial forms under observation belong to the same species. With the exception of

⁷ Potato cultures were obtained through courtesy of H. H. Whetzel, of Cornell University, Ithaca, N. Y.

⁸ Cultures from lettuce by Ivan C. Jagger and W. S. Beach; from sunflower, by C. E. Owens; and from yarrow, by H. A. Edson.

⁹ Culture by G. H. Godfrey.



Slices of cucumber inoculated with four species of *Sclerotinia*, showing growth of the fungi, and the type of decay produced at room temperature

- A.—Decay produced by *S. libertiana*
- B.—Decay produced by *S. intermedia*
- C.—Control
- D.—Decay produced by *S. ricini*. Note heavy brown growth of conidia stage
- E.—Decay produced by *S. minor*, showing more rapid and complete decay of the cucumber and formation of sclerotia

S. ricini, which produces a darker-colored decay than that of the other species, there is not enough constant difference in texture and color of the lesions to justify any attempt to differentiate between the different decays produced by the different species.

TEMPERATURE STUDIES

GROWTH ON CULTURE MEDIA

In cultural studies of *Sclerotinia libertiana*, *S. minor*, and *S. intermedia*, the greatest differences in habit of growth have been found

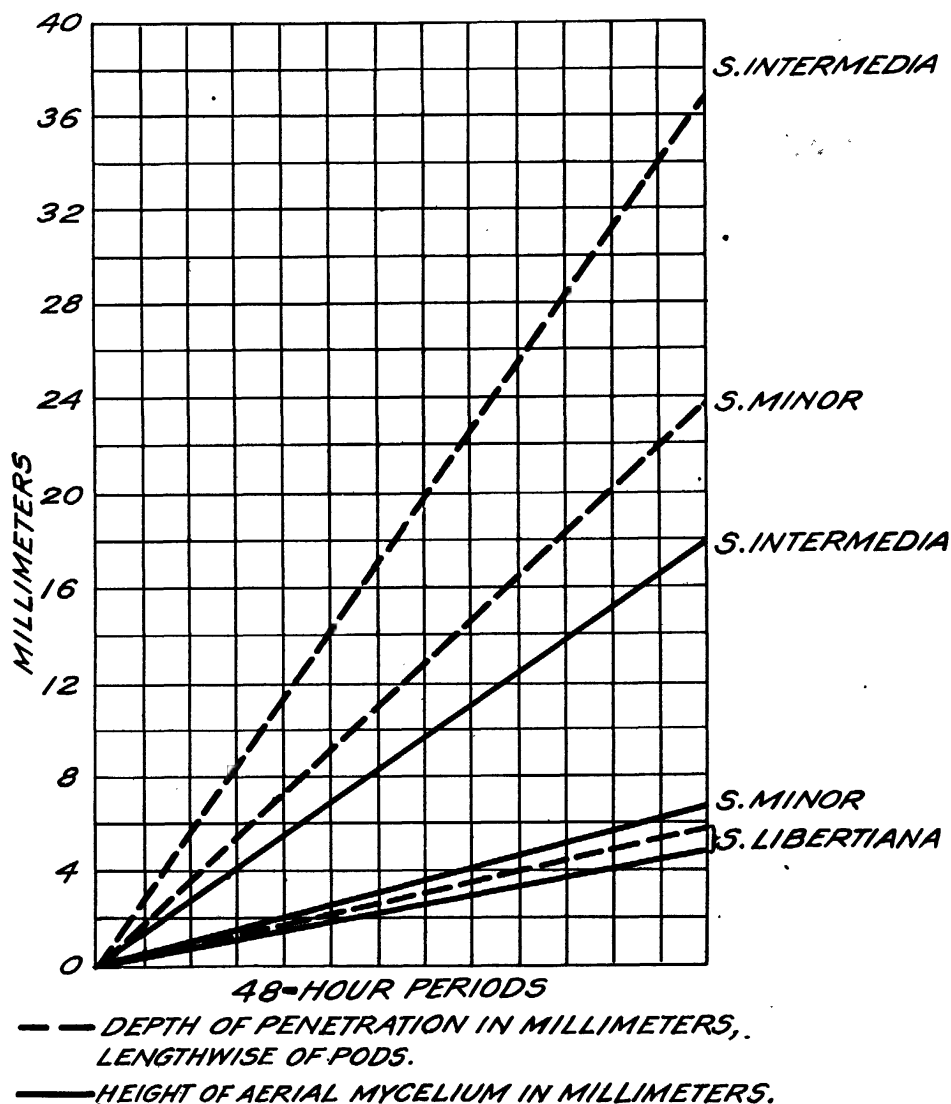


FIG. 1.—Graph showing average radial growth in millimeters of 14 strains of *S. libertiana*, 4 strains of *S. minor*, and 1 strain of *S. intermedia* on potato-dextrose agar at 0° C. during 10-day period

when low temperatures are involved. The mycelium of each species has been found to remain viable after exposure to $-5^{\circ}\text{C}.$, and to make growth on potato-dextrose agar below 0° , but the rates of growth are widely different. This is especially noticeable with *S. intermedia*, which, as a rule, grows about twice as fast as either of the other species. Figure 1 shows the results of an experiment

involving 14 strains of *S. libertiana*, 4 of *S. minor*, and 1 of *S. intermedia*. The cultures were made on potato-dextrose agar. The inoculum in each instance was a small bit of agar containing a young, vigorously growing mycelium. All cultures were held at 15° C. for 12 hours, in order to let the fungi establish themselves, and were then placed in cold storage at 0°. The temperature was carefully checked throughout the experiment, and the range was found to be -0.5 to 0°, as shown by Figure 2.

During the 12-hour period in which the cultures were all at 15° C., *S. minor* and *S. intermedia* made a radial growth of 0.5 mm., while *S. libertiana* grew 1 mm. It will be noted that immediately following storage at 0° the rate of growth of *S. libertiana* was considerably checked. *S. minor* continued at a slightly faster rate than *S. libertiana*, while *S. intermedia* made rapid growth in comparison. At the end of the 10-day period, *S. intermedia* had made slightly more than twice as great a radial growth as either of the other species. This ratio of growth was maintained up to temperatures slightly above 0°. Thereafter these differences become gradually less marked until at 7° each of these species makes approximately the same rate of growth (pl. 6, C). At all temperatures above 7°, *S. libertiana* gradually makes more rapid headway until when a temperature of 20° is obtained, it grows about twice as fast as either of the other species (pl. 6, A, B, and D).

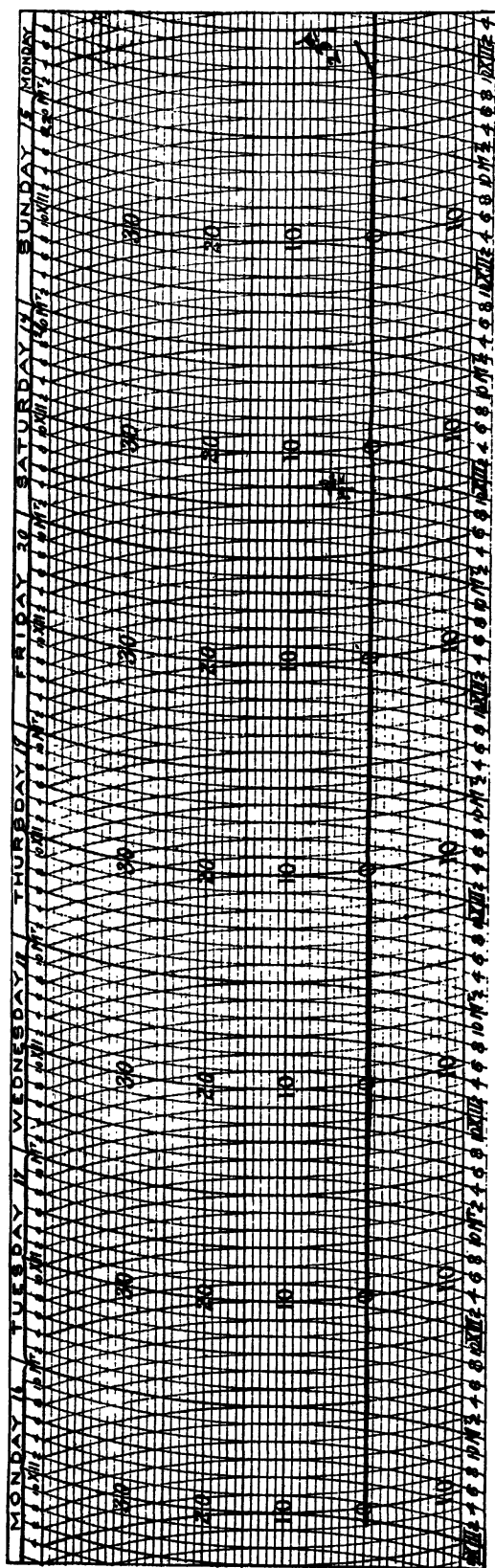


Fig. 2.—Thermograph record showing temperature maintained in 0° C. chamber during experiment to determine rate of growth of *Sclerotinia* species on potato-dextrose agar

Ascospores germinate very readily in sterile water and nutrient broths throughout a wide range of temperatures (pl. 3 C, *a, b, c, d*). Successful germination has been obtained with the strains of *S. libertiana* tested at temperatures as low as 3 to 4° C. (pl. 3, D, *a to g*) and as high as 30 to 31°. Ascospores on the surface of a nutrient agar plate, however, failed to germinate when subjected to a temperature of 30 to 31° for a week. This indicates that the spores can not withstand the drying effect of this high temperature unless they are surrounded with moisture in great abundance.

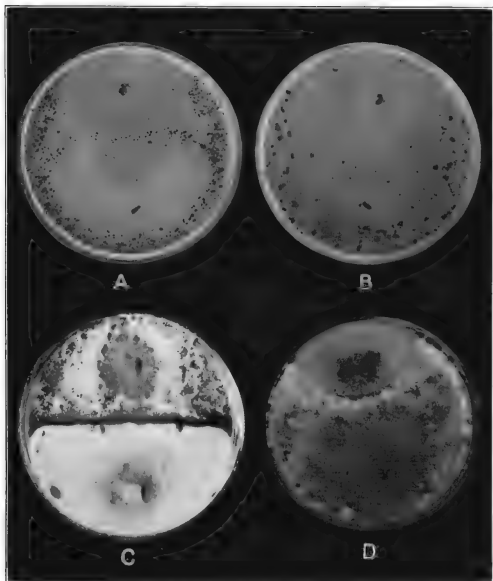
Although no temperature experiments were made with ascospores used as inoculum for infecting plants, it was of interest to the writer to find that the ascospores could withstand such low temperature. "Shooting" ascospores of *S. libertiana*, caught upon a potato-dextrose agar plate which was placed immediately in a refrigerator, retained their vitality after being subjected to a temperature that fluctuated between 5° and -3° C. for 4½ days. No growth was made the first day, and only slight growth was evident within the 4½-day period. However, after the plate was placed at room temperature for 3 days, the surface of the medium was completely covered with the fine, white, cottony mycelium characteristic of *Sclerotinia*.

GROWTH IN HOST TISSUES

From a market point of view, it is of extreme importance to determine the temperature relations of *Sclerotinia*, and to determine whether there is any possibility of controlling decay by low temperatures. So far as the writer has been able to ascertain, the lower temperature limits of infection by *Sclerotinia* have never been determined. In order to obtain information along this line, several inoculation experiments have been carried out in which various hosts were tested.

Figure 3 presents in graph form the results of a typical experiment. Fresh bean pods of the Golden Wax variety were sterilized in mercuric chloride (1 to 1,000) for three minutes, and thoroughly washed in sterile distilled water; each pod was then cut in half and placed in a large test tube containing 3 c.c. of sterile water. The freshly cut ends of these pods were inoculated with young mycelium from potato-dextrose agar plate cultures. After inoculation all cultures were placed immediately in cold storage at 0°. At the end of 26 days the cultures were taken out and examined, both for penetration of the host tissues and aerial growth of the fungus. In each case it was found that the depth of penetration of the fungus was somewhat greater than the corresponding height of the aerial growth. *S. libertiana* penetrated along the long axis of the pod a distance of 6 mm., while *S. minor* and *S. intermedia* penetrated 24 mm. and 37 mm., respectively. The rate of growth as here shown is somewhat slower than that of the same fungi on potato-dextrose agar. However, the same relative positions are held as regards differences in growth rate among the three species.

This experiment taken together with others of similar nature, seems to show conclusively that it is possible for vegetables to become infected with *Sclerotinia* and for a slow decay to result, even though the product is held at 0° C. In other experiments in which beans, peas, and carrots were used in inoculations at 3° to 4°, decay took



Cultures of *Sclerotinia libertiana*, *S. intermedia*, and *S. minor* on potato-dextrose agar plates, showing differences in rate of growth at 7° C. and 20°

A.—*S. intermedia* (above) and *S. minor* (below), planted together, and held at 20°. *S. intermedia* did not form any sclerotia, and its growth was slow in comparison with *S. minor*

B.—Two strains of *S. intermedia*, planted together, and held at 20°. The strain from salsify (above), and the strain from carrot (below), grew at equal rates and met in the center of the plate, each forming sclerotia

C.—*S. intermedia* (above) and *S. libertiana* (below), grown together, at 7°. At this temperature each species grows at about the same rate

D.—*S. intermedia* (above), and *S. libertiana* (below), showing the latter growing about twice as fast as *S. intermedia* at 20°

place much more readily, but developed to no marked extent until after the products had been held for about two weeks. In every case *S. intermedia* grew fastest and produced most decay at the temperatures just mentioned.

Jagger (18) found, in his studies of *S. minor* on lettuce, that this species produced a more rapid decay than *S. libertiana* and that the mycelium was less conspicuous in the decaying plants. In the writer's experience these observations hold true for most of the hosts

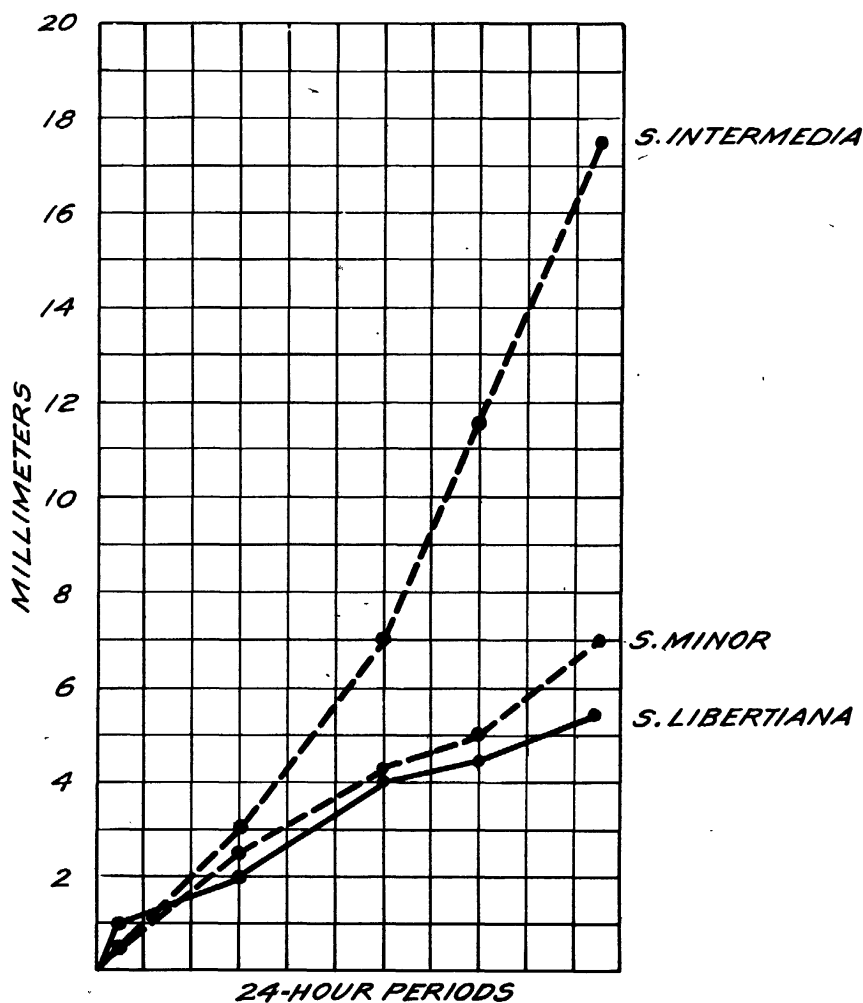
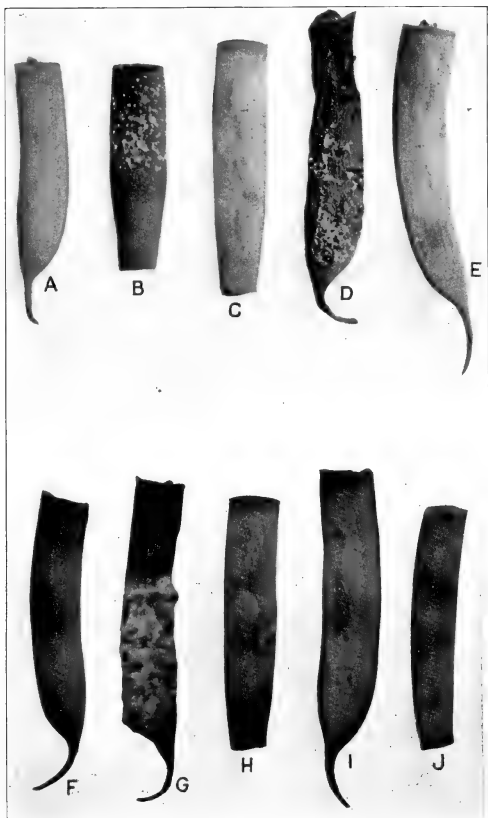


FIG. 3.—Graph showing growth of three species of *Sclerotinia* on bean pods when held at 0° C. for 26 days

on which cross-inoculation studies have been made, with a few exceptions, which might be explained by temperature influences or variations in host susceptibility. In an experiment in which fresh bean pods were inoculated on freshly cut surfaces and placed in test tubes containing 3 c. c. of sterile water and held at 20° C., the pods which were inoculated with *S. minor* decayed faster than those inoculated with either *S. libertiana* or *S. intermedia*. Parallel inoculations of the above experiment were held at 7°, and, as shown in Plate 7, A, E, F, J, very good control was accomplished during this 10-day experiment.



Fresh bean pods inoculated with *Sclerotinia libertiana*, *S. minor*, and *S. intermedia*, showing growth of these fungi and decay produced at 20° C. as compared with that produced at 7° during a 10-day period

A, B.—Development of a strain of *S. minor* from lettuce, at the temperatures indicated (A=7° and B=20°)

C.—Control

D, E.—A strain of *S. minor* from sunflower. Note complete decay and the formation of sclerotia at 20°, and the absence of decay at 7°

F, G.—*S. libertiana* growth at the indicated temperatures. At 20° decay is not as rapid and complete as that produced by *S. minor*. At 7° the fungus is just beginning to make headway within the 10-day period

H.—Control

I, J.—Slow decay produced by *S. intermedia* at 20°, and a slightly more rapid decay at 7°, as compared with *S. minor* and *S. libertiana* at the same temperature

At temperatures ranging between 27° and 30° C. a large proportion of inoculations were negative. While parallel agar cultures carried at these temperatures showed growth and development, the progress made was comparatively slow and the appearance of the culture was more or less abnormal, the growth of *S. intermedia* being more inhibited than either *S. minor* or *S. libertiana*. With the exception of *S. intermedia*, which stops growing at 30° to 31°, each of these cultures has shown ability to make slight growth on potato-dextrose agar at 32° to 33°.

To determine the ability of these fungi to produce infection at high temperatures, freshly cut young carrot roots were inoculated and placed in a high temperature chamber. For the nine-day period of the experiment the temperature varied between 31° and 34°, with a mean of 32°. No growth was found in any culture. After these cultures were held for five days at room temperature there was no evidence of life. Control cultures run at room temperature showed vigorous growth and decay of the carrots within a few days, so there can be no question as to the viability of the organisms. Apparently, these fungi are unable to grow enough to establish themselves in the tissues of the host plants at a temperature of 32°.

The amount of Sclerotinia decay found under transit, storage, and market conditions is necessarily dependent upon the extent of infection in the field. Once the product is contaminated with spores or mycelium of the fungus and has started on its way to market, the most effective method of controlling the amount of decay is by refrigeration. As experiments have shown, however, this method of control is somewhat limited. Under the present methods of refrigeration a car which maintains a temperature of 40° F. at the bottom and 45° to 46° at the top is considered a good refrigerator, but unfortunately Sclerotinia will develop and cause decay at these temperatures. The writer's experiments show that *S. libertiana*, *S. minor*, and *S. intermedia* can infect freshly made wounds in suitable host plants and cause appreciable decay at these temperatures. In fact, the experiments conducted in cold storage show quite clearly that infection of freshly wounded tissues can take place even at 32° F. It is therefore manifestly impossible to get absolute control of these fungi by any practical method of refrigeration which would not also be disastrous to the produce. From a practical viewpoint, however, the experiments show that although incipient infections or contaminated wounds present at shipping time may develop decay at a temperature of 40° to 45° F., little spreading from one plant to another or development of decay in unwounded tissues is to be expected during the ordinary transit period.

The most effective method of preventing Sclerotinia decay in transit is, of course, to prevent the loading of infected produce or badly wounded produce that has had a chance to become contaminated. Clean, carefully graded vegetables will carry well under present methods of refrigeration at temperatures of 40° to 45° F. The precooling of vegetable produce is advisable whenever possible, however, on account of the fact that without precooling it takes from one to three days in the refrigerator car to reduce the temperature of the product to a point which is really effective in controlling Sclerotinia decay. Small lesions or contaminated wounds present

at shipping time in vegetables which are not precooled may therefore develop considerable decay during the first few days of transit in spite of the fact that the car may show a perfect icing record and a temperature of 40° to 45° F. on arrival at market.

It is rather difficult to control *Sclerotinia* decay in the field on account of the fact that the soil soon becomes heavily seeded with sclerotia if the same crop is planted year after year, and it is often difficult to find profitable truck crops to rotate which are not also subject to attack by this fungus. The soil may be sterilized by means of heat or chemicals, but this is practicable for only small plots and greenhouses. The selection of the more upright varieties of lettuce and the practice of general field sanitation, together with two or three year crop rotation system, seems to be the best recommendation for the control of *Sclerotinia* diseases in the field.

In summarizing all studies and experiments made upon the various *Sclerotinia* cultures isolated from vegetable produce on the market, with the exception of *S. intermedia*, which was discovered during the progress of these investigations, and one other undetermined strain, all cultures have shown morphological as well as physiological characteristics of *Sclerotinia libertiana* Fckl. More than 90 per cent of all *Sclerotinia* cultures isolated by the writer in the last four years belong to this species.

SUMMARY AND CONCLUSIONS

Sclerotinia libertiana Fckl. and related species cause great damage to truck crops during transit and storage, as well as in the field.

All strains of *Sclerotinia* under observation produce a soft, watery, odorless type of decay of the host plants.

Observations on the market indicate that produce harvested during wet weather is especially susceptible to *Sclerotinia* infection and decay during transit.

Vegetable produce having little or no visible decay at time of shipment may show a high percentage of infection and decay upon arrival at market.

S. libertiana is not limited to vegetable crops, but may also attack fruits. The manner of growth of the host and the opportunities for infection seem to be the important limiting factors.

From 20 to 50 per cent of the cars of carrots, lettuce, and celery which the Bureau of Agricultural Economics, United States Department of Agriculture, is called upon to inspect show decay caused by *Sclerotinia*. The amount of infection often averages higher than 50 per cent, and in some instances 100 per cent of the shipment is affected.

Morphological and cultural studies show that there is no difference between cultures arising from sclerotial and mycelial plantings, other than variations in rate of growth.

Cultural studies on agar indicate that at temperatures approaching the minimum for the strain, the sclerotia formed are likely to be larger than normal for room temperature. At temperatures nearing the maximum for the fungus the sclerotia formed are often smaller than normal.

On media favorable for the development of the fungus the sclerotia formed are larger than those formed on unfavorable media.

No definite period of dormancy is necessary to prepare sclerotia for production of apothecia. The ecological factors of moisture, temperature, and light determine apothecial formation.

Small sclerotia are especially susceptible to decay if kept too moist. This is one reason why it is usually much more difficult to obtain apothecia from *S. minor* and *S. intermedia* than from *S. libertiana*.

Morphological studies, including measurements of sclerotial apothecia, asci, and spores, show that all of the large sclerotia, forms found upon the market belong to the species *S. libertiana*.

All strains of *Sclerotinia* under observation produced microconidia to a greater or less extent sometime during their life history.

The amount of available food is the chief factor in determining the time of microconidial production. Media unfavorable for the vegetative growth of the fungus induces a rapid and prolific production of microconidia.

Previous to this time, as far as the writer knows, no one has ever observed germination of the microconidia in the genus *Sclerotinia*. In these studies the writer has been able to induce the microconidia of several strains to germinate.

The experiments herein described indicate that the microconidia do not play an important rôle in the life history of the genus *Sclerotinia*.

Artificial infection of host plants is readily obtained by inoculation with sclerotia and mycelium, under favorable conditions. Moisture and temperature are the important limiting factors in determining infection. Infection may take place through unwounded as well as wounded surfaces.

The more succulent plants and plant parts are penetrated most rapidly, while the woody tissues are a great deal more resistant, thus indicating that the mechanical make-up of the plant may play a part in resistance to *Sclerotinia* infection.

Infection of freshly cut surfaces of turnip and carrot roots, by holding them over "shooting" apothecia, has proved successful. An ascospore dilution in distilled water sprayed over lettuce leaves also gave positive results.

Cross-inoculation studies show that *S. libertiana*, *S. intermedia*, *S. ricini*, and *S. minor* are capable of producing decay in a wide range of vegetables.

None of the *Sclerotinia* cultures were found to be pathogenic to potato tubers. Beet roots were also highly resistant, although a few inoculations produced slight decay.

The host range of the *Sclerotinia* species considered in this study was so wide that it is impossible to separate them on the basis of host infection. The decay produced (*S. ricini* excepted) is so similar that it is not until peculiarities of mycelium or sclerotial growth become evident that the species can be distinguished.

S. minor produced a more rapid decay than any of the other species when inoculated into bean pods and other host plants and held at 20° C.

Inoculation studies as well as plate cultures show that *Sclerotinia* is able to grow and produce infection at temperatures as low as -0.5° to 0° C. Of all strains tested, *S. intermedia* grew fastest and produced most decay at this temperature.

With the exception of *S. intermedia*, which stops growth at 30° to 31° C., all other strains have shown ability to make slight growth on potato-dextrose agar at 32° to 33° C. Inoculation experiments conducted at this temperature proved negative.

Ascospores germinate readily in sterile water and nutrient solutions throughout a wide range of temperatures. Successful germination has been obtained at temperatures as low as 3° to 4° C. and as high as 30° to 31°.

Experiments show that incipient lesions and contaminated wounds will develop decay during transit under refrigeration temperatures of 40° to 45° F., but that development of new lesions and spreading of the fungus from one plant to another during the ordinary transit period will be fairly well controlled. Clean, carefully graded vegetable produce which has been precooled at loading point should arrive on the market in good condition if held throughout the trip at temperatures of 40° to 45° F.

A summary of all observations and experiments shows that more than 90 per cent of the *Sclerotinia* cultures isolated from vegetable produce on the market during the past four years belong to the species *S. libertiana*.

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AGE OF SEEDLINGS AS A FACTOR IN THE RESISTANCE OF MAIZE TO SODIUM CHLORIDE¹

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INTRODUCTION

Much work has been done in recent years in comparing different genera, species, varieties, and strains of plants as to their resistance to the effect of salt solutions. Harter (3),² Kearney and Harter (5) and others have called attention to the opportunity for selecting and breeding the most resistant strains for alkali soils common in the arid portions of the United States, but apparently little has been accomplished along such lines.

The work of the writers, the results of which are presented in this paper, was taken up in 1923 at the suggestion of G. N. Collins, botanist in charge of biophysical investigations of the Bureau of Plant Industry, United States Department of Agriculture, who suggested the water-culture method, whereby great numbers of individuals could be handled and observed in a short time, as a means of segregating individuals showing marked resistance to the effects of the salts contained in alkali soils.

Since sodium chloride is a predominating salt in the southern Arizona valleys,³ it was decided to make use of this salt alone in the preliminary experiments (6). Corn was chosen as the crop to use because of the ease of handling and transplanting the seedlings and its suitability for genetic studies. Extreme variation in tolerance of seedlings of different sizes to the effects of sodium chloride was encountered soon after the investigations were started, and the results obtained in this phase of the work are presented and discussed in this paper.

EXPERIMENTAL METHODS

The seeds were sprouted on a lime-sawdust germinating table (4), and allowed to remain until the radicles had attained convenient length for suspending in the salt solutions, then the seedlings were transferred to perforated aluminum disks which were floated by means of sealed glass buoys on the surface of the solutions contained in enameled milk pans (1).⁴ These culture pans contained 3 liters of the salt solution, and the volume was kept constant by the use of Mariotte flasks suspended above each pan. In all of the work prior to May 22 the seedlings were assorted according to the length of the primary radicle, and 100 plants of uniform radicle length were placed

¹ Received for publication Jan. 7, 1925; issued December.

² Reference is made by number (italic) to "Literature cited," p. 640.

³ The results of analyses by the Bureau of Soils, U. S. Department of Agriculture (6), indicate that sodium and chlorine comprise on an average 56.3 per cent of the total alkali salts in the soils of the U. S. Field Station at Sacaton, Ariz. (Na 31.9 and Cl 24.4).

⁴ The writers gratefully acknowledge the helpful assistance rendered by J. F. Breazeale, associate biochemist in the Office of Western Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture, who performed work along similar lines in 1921 and 1922.

on each disk. It soon became evident that the number of seedlings surviving the salt solution increased somewhat in proportion to the length of the radicles. In the effort to determine the limit of age or size influence on resistance, tests were made with seedlings which had been allowed to develop primary roots 100 to 125 mm. long before subjecting them to the solutions. After finding increased resistance, even at this advanced stage, it became necessary to resort to some other method of classification according to size in order to work with still older seedlings, since at this stage many secondary seminal roots had developed and difficulty was encountered in handling and measuring the roots. It was found that the stage of development of the plumule afforded a very satisfactory means of classing the seedlings at this age, and no measurements were necessary. After May 22 all classification was based on the plumule development, and the radicle length was disregarded.

In the preparation of the seedlings for testing in groups according to stage of plumule development, they were sorted and transferred to culture pans containing tap water as soon as the radicles of the youngest group were of sufficient length to balance the seeds well on the perforated disks. It was usually possible at this time to sort fairly accurately; however, when the desired stage of development was reached they were again classified according to the extent of plumule development and then placed in a salt solution of the desired concentration.

During a greater part of the investigation the seedlings were allowed to remain in the salt solution for 72 hours, but it became evident when working with seedlings in the plumule stage, and especially during warm weather, that this period frequently was too short to bring to the death point more than 50 per cent of the individuals, even in the high concentrations used, and the period was increased to 96 hours.

After subjecting the seedlings to the solutions for the 96-hour period, the number of individuals which apparently had survived in each pan was recorded and the live plants were transferred in groups to other pans containing tap water. Another count was made 72 hours later, and plants which had failed to resume growth of roots or plumule in the water were classed as dead. The extent of the green coloring in the basal portion of the coleoptyle afforded an easy means of distinguishing those seedlings which were likely to overcome their injuries when removed to tap water, and it was seldom that more than 4 or 5 per cent of seedlings of the first selection were later eliminated.

After working with varied concentrations for several months the writers became convinced that the stage of growth of the seedlings was a greater factor in determining the toxic limits of the plants than small differences in the amount of salt in solution. It was therefore decided to investigate this phase of the problem, and a concentration of 22,000 parts per million of sodium chloride was adopted as a standard for further study.

In view of the methods employed in this experiment it is obvious that the results are not comparable with those obtained by most of the previous investigators, some of whom have sought to fix the maximum tolerance or toxic limits of various plants for alkali salts. In previous investigations effort apparently has been made to deter-

mine the highest concentration of salt to which a plant can be exposed without showing injury to any of its organs or without seriously affecting its ordinary behavior or growth. In this experiment the purpose was to kill off a greater part of the plant population in order to isolate those individuals which showed a marked resistance. It will be seen that salt concentrations used in this experiment are considerably higher than those used by most of the other investigators, who have seldom carried the concentration to the point where the entire plant was killed.

RESISTANCE OF SEEDLINGS WITH DIFFERENT LENGTHS OF RADICLES

At the beginning of the experiment seedlings were used which had developed radicles only 25 to 50 millimeters long with plumules still encased in the coleoptyles. It soon was discovered that seedlings of this size were unsatisfactory for use in the higher concentrations of salt, for, where the percentage of salt was as great as 1.8 per cent, no further elongation of the radicle was observed after a few hours in solution. A few of the seedlings developed a hardened bulbous enlargement just behind the tip of the radicle, and in such cases the root remained turgid and healthy but without further growth of radicle or coleoptyle. On most of the seedlings, however, the radicle became flaccid during the first 24 hours in the salt solution, and at the end of 72 hours many of the primary roots had sloughed off, leaving only a stub attached to the mesocotyl (2). At the end of this period the coleoptyles of many of the seedlings had assumed a yellowish color and showed signs of decomposition. A few seedlings however, after losing their radicles began to develop secondary roots from the base of the mesocotyl or laterally from the remaining stub of the radicle. In such cases there occurred a quick response in growth of the plumule, and development was continued though slowly for several days while exposed to the salt solution. Inasmuch as the primary or axial root soon dies under field conditions in the early stages of development of the maize plant, it was decided that the most valuable test of resistance should occur after the brace roots or permanent roots had begun to function.

The percentage of seedlings with radicles of various lengths which survived after 72 hours in the salt solutions are shown in Table I.

TABLE I.—Percentage of maize seedlings surviving after 72 hours in salt solution, when the primary radicles were at different lengths between 25 and 100 millimeters

Date	Percentage of sodium chloride	Length of radicle (percentage of seedlings surviving ^a)					
		25 mm.	38 mm.	42 mm.	51 mm.	88 mm.	100 mm.
1924							
Mar. 29.....	2.0	-----	11.7±2.33	-----	-----	-----	-----
Mar. 31.....	2.2	-----	11.2±2.18	-----	-----	-----	-----
Apr. 5.....	2.0	6.9±1.76	-----	-----	-----	-----	-----
Apr. 6.....	2.0	-----	6.9±1.76	18.4±2.48	-----	-----	-----
Do.....	2.2	-----	-----	14.8±2.33	-----	-----	-----
Apr. 14.....	2.2	3.3±1.20	11.2±2.13	11.4±2.45	-----	-----	-----
Apr. 21.....	2.2	-----	4.9±1.47	-----	8.6±2.00	-----	-----
May 22.....	2.2	3.1±1.169	8.9±2.04	-----	12.8±2.35	22.8±2.84	23.1±2.84

^a The irregular growth of the radicles made it impracticable to select a sufficient number of seedlings of the various radicle lengths to represent each group for comparison on the same day.

Since it was found impracticable, on account of fluctuating weather conditions, to obtain consistent results from seedlings selected on different dates, it is suggested that the data in Tables I, II, and III be studied and compared under one date at a time.

The heaviest mortality occurred where the radicles were only 25 millimeters long. In this stage the cotyledon was just pushing through the seed coat and only the primary root had emerged. The greatest number of plants survived from stages where the radicles had attained considerable length and where the plumule had ruptured the tip of the coleoptyle.

In testing the effect of different concentrations on the seedlings it was noted that a more rapid plumule growth often developed in concentrations of 18,000 to 20,000 parts per million than in weaker solutions. It was discovered that this could be attributed to the rapid decay of the primary radicle and the prompt production of permanent roots to carry on the development. In order to test the rapidity of response to the destruction of the primary radicle, several comparisons were made between pan cultures of seedlings in part of which the radicles had been excised with a scalpel about 6 millimeters from the base of the mesocotyl and others which received no mutilation. The percentage of seedlings remaining alive after 72 hours in the salt solutions is given in Table II.

TABLE II.—Percentage of maize seedlings with excised and normal radicles surviving after 72 hours in salt solution (22,000 parts per million)

Date	Percentage of seedlings surviving	
	Radicles excised *	Radicles not excised
1924		
Apr. 6.....	15.0±2.41	2.5±1.05
Apr. 14.....	40.8±3.37	14.8±2.41
Apr. 21.....	21.0±2.75	5.7±1.62

* Seedlings having radicles approximately 40 millimeters long were used, and approximately 100 plants of each treatment were used on the respective dates.

The results in Table II seem to indicate that any form of injury to the primary radicles of the seedling stimulates the early production of permanent roots, and these latter appear to enable the seedling better to withstand salt solutions than do the primary roots.

The results also indicate the necessity of strictly standardizing any method of testing seedlings for alkali resistance. It is obvious that any injuries to the seedling roots in handling might easily influence the number of plants selected as resistant where the growth of the plumule was used as a criterion.

The behavior of the seedlings with excised radicles suggests that the apparent resistance exhibited by some very young seedlings whose primary radicles remained turgid with hardened bulbous tips, as previously described, may be of little significance as their response in growth when transferred to water was not nearly so rapid as the surviving plants with excised radicles.

RESISTANCE OF SEEDLINGS WITH VARIOUS STAGES OF PLUMULE DEVELOPMENT

After finding that the older seedlings could be more easily and accurately classified by the stage of plumule development than by the length of radicles, the seedlings, in all of the later experiments after being sorted and placed on the disks, were allowed to remain in tap water until the youngest group reached the stage when the leaf roll had just emerged from the tip of the coleoptyle, then the disks, each containing 100 plants, were floated on the salt solutions. The percentage of seedlings of different sizes which survived an exposure of 96 hours to the salt solution (22,000 parts per million) is shown in Table III.

TABLE III.—Comparison of the percentage of seedlings at different stages of plumule development surviving after 96 hours in salt solution (22,000 parts per million)

Date	Well out of coleoptyle ¹	Just prior to unfurling first leaf	First leaf unfurled	Second leaf appearing
1924				
May 31	15.2±2.67	53.5±3.11		7.0±2.35
Do.	19.0±2.65			
Do.	7.0±1.72			
June 3.	7.2±1.72	61.0±3.29		
Do.	20.0±2.69	44.0±3.35		
June 9.		17.3±2.53	10.0±2.02	9.4±1.99
June 15.		26.0±2.96	10.0±2.02	3.0±1.25
Do.		21.0±2.75		
June 25.	10.9±2.11	25.3±2.92		6.4±1.63
Do.	20.0±2.69			
Do.	35.0±3.22			
July 1.		42.2±3.23	15.3±2.59	12.3±2.44
Do.		32.5±3.32		
July 6.		32.0±3.14		8.0±1.83
Do.		25.0±2.92		
July 22.	16.7±2.53	31.0±3.12	5.5±1.82	9.4±2.01
Do.		54.5±3.07		

¹ The radicles at this stage were from 60 to 100 millimeters long. The other three groups represent a more advanced stage of growth than classification shown in Table II.

From the data in Table III it is apparent that there is a critical stage in the development of the seedlings at which their metabolic functions are less interrupted by toxic salts than at any other time. This stage occurs about the time when the first leaf has pushed out of the coleoptyle for 10 or 15 millimeters, and but a few hours before it begins to unfold into blade form. An explanation for this might be found by determining the rate of reduction of the stored food in the endosperm, and the stage at which the seedling begins to decline in vigor when not provided with nutrient material from an outside source.

While the data shown in Table III are far from consistent, especially when comparing results obtained from series of plants handled on different dates, they seem, however, to indicate clearly that the stage of development is of primary importance in determining the relative resistance of seedling to toxic salt solutions, and that the most resistant stage to sodium chloride is just prior to the unfolding of the first leaf (fig. 1, stage No. 2). When the data are studied under one date at a time, it will be observed that there are no discrepancies; all the results indicate an increasing resistance as seedlings develop after first sprouting, until the leaves begin to unfold, and then a diminishing resistance as they become older.

WEATHER CONDITIONS A FACTOR IN ALKALI TOLERANCE

The wide fluctuations in the percentage of survivors on different dates were apparently due to changes in weather conditions, while the seedlings were exposed to the salt solutions. No detailed notes were taken on the daily behavior and appearance of the seedlings in relation to weather conditions, but it was noted when making the counts that the number of survivors was likely to be small if exceptionally high temperature in combination with high wind velocity and low relative humidity prevailed during a considerable part of the time when the seedlings were being tested. It was also observed that when cloudy weather, with high relative humidity prevailed during one or more days of the test, the number of surviving seedlings usually increased.

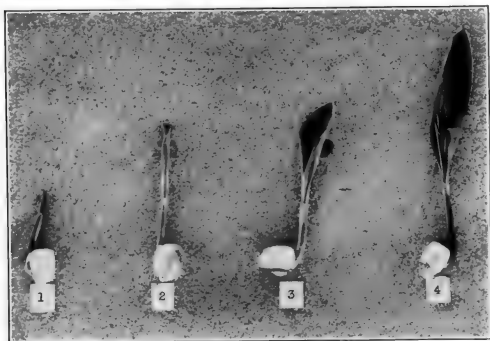


FIG. 1.—The four stages of plumule development at which maize seedlings were subjected to concentrated sodium-chloride solutions. Stage No. 2 illustrates that of greatest resistance, and stage No. 4 that of least resistance

The mean maximum and minimum temperatures, relative humidity, and wind velocity, during the three and four day periods while the seedlings were being tested are given in Table IV.

The data in Tables III and IV indicate that the high relative humidity occurring during the period May 28 to June 3 apparently affected an increase in the seedlings which were counted on May 31 and June 3. During June 6 to 9 there occurred a period of low relative humidity accompanied by unusually high wind velocity and its effect is reflected in the relatively small percentage of survivors recorded on June 9.

There were indications also that seasonal changes influenced the resistance of the seedlings to the salt solutions, and some adjustments were made in the method in order to meet these conditions. It was found that the plants developed more rapidly and showed distress more quickly during the hot months of summer than during the spring and autumn.

TABLE IV.—Mean maximum and minimum temperatures, relative humidity, wind velocity, and total precipitation, during the three and four day periods while seedlings were exposed to sodium-chloride solutions

Period	Mean maximum temperature	Mean minimum temperature	Mean relative humidity	Mean wind velocity	Rainfall during period
Mar. 27 to 29.....	66	43	54	2.4	0.44
Mar. 29 to 31.....	66	41	58	1.9	-----
Apr. 3 to 5.....	79	46	48	2.1	.03
Apr. 4 to 6.....	80	50	64	3.6	.13
Apr. 12 to 14.....	78	51	60	2.6	-----
Apr. 19 to 21.....	90	44	33	2.1	-----
May 20 to 22.....	98	58	31	2.1	-----
May 28 to 31.....	91	61	49	2.9	.51
May 31 to June 3.....	98	61	51	1.8	-----
June 6 to 9.....	98	56	29	3.7	-----
June 12 to 15.....	109	70	38	1.9	-----
June 22 to 25.....	106	67	42	1.6	-----
June 28 to July 1.....	106	79	45	2.0	-----
July 3 to 6.....	101	75	56	2.2	.04
July 19-22.....	101	62	40	1.8	-----

SUMMARY

The use of water cultures in breeding alkali-resistant strains of maize, by selecting individual seedlings which survived strong salt solutions, was studied and various modifications were tested.

The stage of development of the seedlings was found to be an important factor in determining the amount of salt in solution which they were able to withstand.

In testing the youngest stages of seedling development, using various lengths of radicles as a basis of grouping, the resistance increased up to certain limits in proportion to the length of the radicles. The greatest number were killed by strong salt solutions when the radicles were 25 millimeters long, and the smallest number when the radicles were 100 millimeters long.

The extent of the development of the plumule was found to be the most satisfactory indicator for preparing uniform groups of seedlings in the older stages of growth.

The stage at which the older seedlings were most resistant to sodium chloride was just prior to the unfolding of the first true leaf.

Young seedlings with short radicles and undeveloped plumules were found to be most susceptible; the oldest plants with two seed leaves were the next in susceptibility; and the intermediate stages were the most resistant.

Seedlings whose radicles were destroyed earliest by the salt solution made the most rapid plumule growth, due to the rapid development and early functioning of the permanent roots.

Artificial excision of the radicles increased the resistance of the seedlings to a marked degree, causing a prompt and comparatively rapid development of permanent roots and plumule, even while exposed to a strong salt solution.

It was found impossible to obtain consistent results when comparing the behavior of seedlings subjected to salt solutions on different dates, the resistance of the seedlings apparently being influenced profoundly by changes in weather conditions.

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ORGANIZATION OF THE TELIAL SORUS IN THE PINE RUST, *GALLOWAYA PINICOLA* ARTH¹

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INTRODUCTION

The short-cycled pine rust now commonly referred to as *Gallowaya* (2)² was first reported under the name *Coleosporium pini* by Galloway (9), who later (10) described its effects on the host *Pinus virginiana*. Although he was not fully aware of the exact nature of the germination of the teleutospores, he figured in some detail various stages in the development of the elements of the sorus and brought out some of the most characteristic features of the fungus. The writer was enabled to make a further study of the fungus from material furnished by W. W. Diehl, who collected quantities of the rust for him in the vicinity of Washington, D. C. It will be shown that there is formed a distinct and persistent peridial buffer structure which functions in rupturing the leaf tissues overlying the young sorus and that, following cell fusions, teleutospores are borne in chains. The spores are not sessile in the sense that only one spore is cut off from a basal cell as in *Coleosporium*. Neither does the basal cell bud to form the spores as in *Puccinia*.

THE GAMETOPHYTIC ELEMENTS

As no one had questioned the results of Galloway's infection work in demonstrating that the rust is short-cycled, it was to be expected that the cells of the mycelium in the pine leaf would be uninucleated. This is clearly the case. The hyphae are readily stained, although the waxy nature of the young sorus renders it difficult always to get good fixation of later stages. Each cell contains a single nucleus (fig. 1, B).

Vestigial spermogonia are to be found occasionally, but the host tissues above them (pl. 1, A) are not fully ruptured and spermatia are seldom formed. The few spermogonia seen in the sections were located beneath stomata. This may be of no significance, as they are rather broad and the stomata are scattered along parallel lines on the flat side of the leaf. The web of hyphae just below the base of the primordium is a little denser than that which is to produce a telium. The end cells which are somewhat enlarged send out smaller branches which converge slightly so that they appear to be directed toward the stomatal opening. Galloway's Figure 10 (10) might very well have been drawn from a section of a spermogonium.

¹ Received for publication Nov. 13, 1924; issued December, 1925.

² Reference is made by number (italic) to "Literature cited," p. 651.

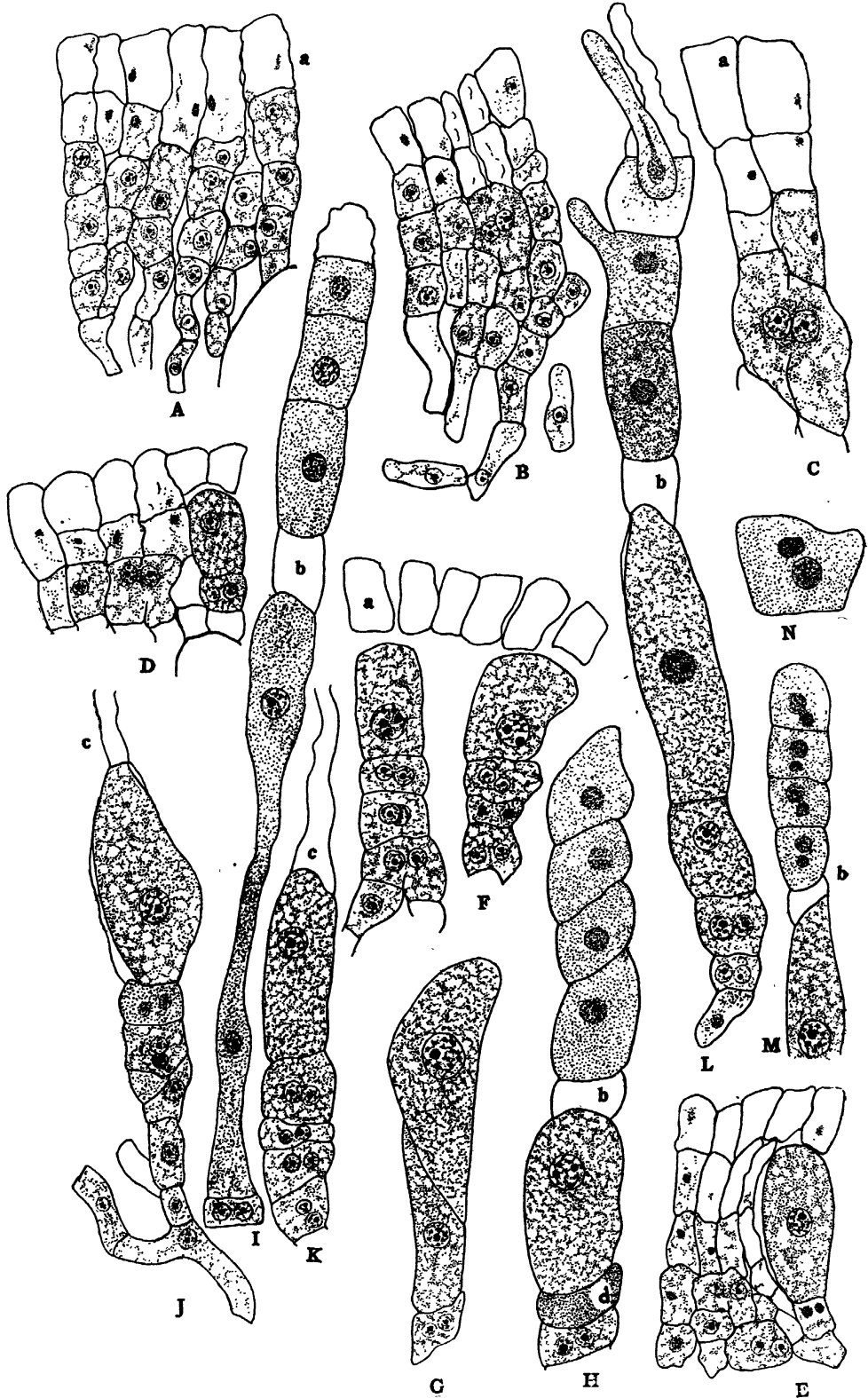


FIG. 1.—A, young primordium before cell fusions; B, first cell fusions, two sterile cells above; C, cell fusion, three sterile cells above; D, first mature spore; E, margin of sorus, fusions at different levels; F, chains of young spores, "peridium" (a) above; G, oblique cell divisions; H, I, basidia supported by elongated part of cell wall (b); J, K, spore chains in old sorus, stalklike remains of spore above; L, typical spore chain; M, N, basidial cells with additional body in each near nucleus. (C, L, and N are more highly magnified, and M and E less highly magnified, than the others.)

THE PERIDIAL BUFFER TISSUE

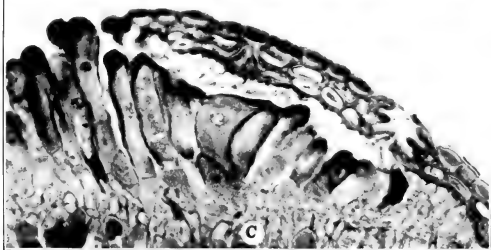
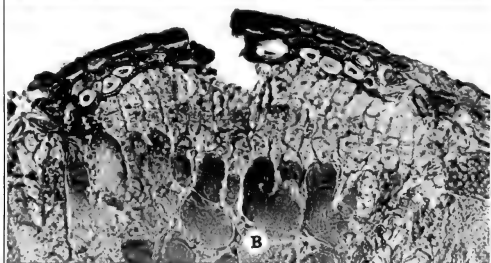
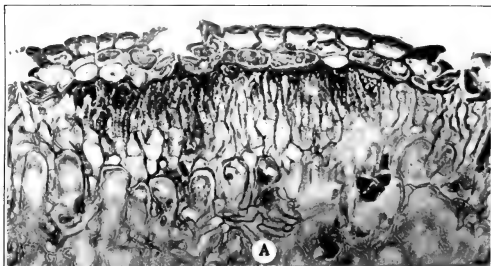
There is very little intermingling or intertwining of hyphae as a preliminary to the formation of the telial sorus. The hyphal complex below is usually no denser than it is in other parts of the leaf. The hyphae push out between the mesophyll cells and widen suddenly just before reaching the ends of the large lobes of these cells. Since the hyphal branches grow straight out against the hypodermal tissue in a solid rank, the epidermis and underlying tissue are stretched and pushed outward. Each chain in the palisade of fungus tissue is composed of four or five uninucleated cells (fig. 1, A). The buffer effect is considerably increased as the terminal cells lose their granular cytoplasmic contents and elongate (fig. 1, C, *a*). The propriety of calling the layer of terminal cells which persists a peridium is discussed later.

CELL FUSIONS

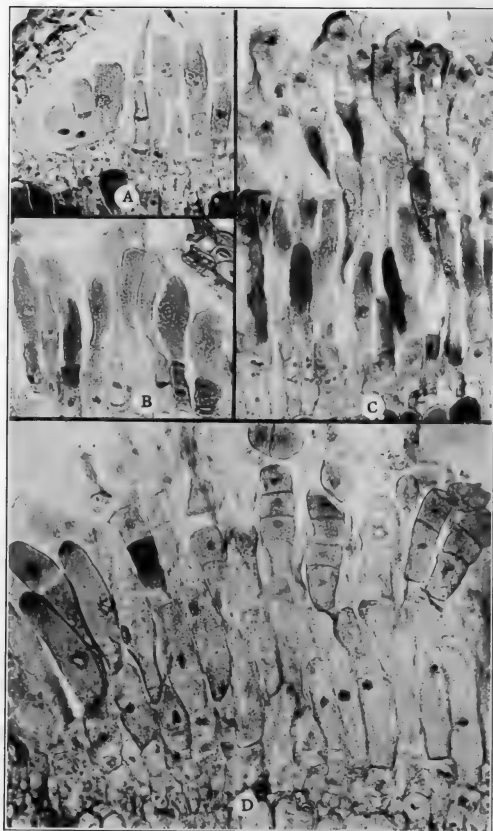
Up to this time there is nothing in the appearance of the cells of the primordium to indicate exactly where cell fusions are to occur. Soon after the epidermis is ruptured, however, the sorus takes on quite a different appearance. Cell fusions which are readily distinguished are seen along a line beneath the point of rupture (fig. 1, B to D). The fusing cells are the second or third cells from the outer ends of the chains, and are therefore intercalary. The writer has not seen fusions between subterminal cells, but this may sometimes occur. Neither are the fusions all on the same level (fig. 1, E). There are more frequently two sterile cells in the chains above the fusing cells (fig. 1, B and D). The conjugations are more readily seen in sections that show the gaps between the lobes of the mesophyll cells. The adjacent walls of the fusing cells quickly disappear, and the two nuclei come closer together (fig. 1, C). The cell thus formed enlarges but its cytoplasm becomes only slightly less vacuolate. At the same time the buffer cells immediately above collapse. The terminal cells of the chains, however, retain their form and persist indefinitely as a sort of peridium, or until cast off with fragments of the ruptured leaf tissue above (pl. 1, C).

CATENULATE SPORES

The fusion cell quickly divides, and the upper daughter cell develops a very dense cytoplasm so that it takes the stains readily. The fusion cell still remains vacuolate. As the sorus ages and increases in size, the buffer cells at the margin elongate correspondingly. The digestive effect which the newly formed spore cells have on this tissue is strikingly demonstrated by the way the fertile layer broadens laterally (pl. 2, A, and fig. 1, E). Although the subterminal cells in the chains are completely destroyed, the terminal cells, at least the outer ends of them, persist, as noted above (pl. 1, C). From this time on, the number of binucleated spore cells cut off in the chain above each fusion cell increases. At the margin, nuclear fusions may occur in the second or third spore of the chain (fig. 1, E); while at the center, there may exist three or four binucleated potential spore cells, above which there are one or two spores, considerably elongated and rich in cytoplasm, but still binucleated (fig. 1, K). Above these the spores are much longer and uninucleated (fig. 1, J).



A.—Spermatogonium of Gallowaya
 B.—Telial sorus before cell fusions
 C.—Sorus showing mature spores, and remains of peridial buffer tissue
 (Same magnification in the three figures)



A.—Chains of spores and "peridium" at margin of a sorus
 B.—Chains of spores in a young sorus
 C.—Spores with stalk-like lower ends, in an old sorus
 D.—Spore chains terminated by mature basidia
 (Same magnification as in Plate 1)

to L). It would seem that there can be no question that the spores of *Gallowaya* are catenulate, since it is possible, in a sorus sufficiently developed, to trace the chain upward from the "two-legged" fusion cell below to the germinated spore above (fig. 1, I and L). Ordinarily, then, one could say that at the middle of the mature sorus the chain of cells may be composed of a fusion basal cell; two to four short vacuolated binucleated cells; one or two elongated binucleated spores rich in cytoplasm; one or two long uninucleated spores; and the terminal germinated spore in the form of a four-celled internal basidium (fig. 1, L).

INTERCALARY ELEMENTS

A feature which is brought out more or less diagrammatically in Figure 1, L, *b*, is well shown in Plate 2, D. In some of the chains of cells there is nothing to suggest that these intercalary structures are cells. At H, however, in Figure 1, *d* is a cell which is clearly degenerating, so that one might think that both *b* and *d* are real intercalary cells. C. R. Orton has suggested to the writer that if such were the regular order of development throughout, it might be concluded that the sorus in *Gallowaya* is a modified aecidium. Such a form would round out an evolutionary series nicely. Until further evidence is found, however, the writer is compelled to consider this intercalary structure, *b*, as formed merely by the elongation of the lower end of the lower cell of the basidium. The wall seems to become separated from the cytoplasmic contents above and elongates in the form of a stalk, pushing the basidium still farther outward. In an aecidium, for example, when the spore is formed, its sister cell is usually sacrificed to serve, as some hold, merely as a disjuncter, or, as others might claim, to make available sufficient food for the maturing of one of the pair. Where the upper daughter cell degenerates and the lower one becomes a spore, the first of these views could not be held. In some cases the intercalary cell elongates to perform the function of a stalk. In *Gallowaya*, some of the true spores fail to develop, and they may then elongate greatly and function as stalk elements. This intercalary portion seems to be simply the swollen end of the cell wall.

Just how many of the cells in the chain function as spores is problematical. Typically, at any given time only the terminal ones do germinate. After the discharge of the sporidia the walls of the basidium collapse and disappear. Following this there is a sudden stretching or elongation at the lower end of the next spore. There appears to be a limit to the amount of elongation this spore can undergo before processes of disorganization begin. The next spore below, by elongating also, helps to bring the new terminal spore into a favorable position as regards its competitors (fig. 1, I). There is no question that as the sorus ages the lower spores in the chains never succeed in functioning as spores. They are, however, called upon to serve a very useful purpose, for it is only by their sacrifice in excessive elongation that the spore above can be brought into a position favorable for the discharge of its sporidia (fig. 1, I to K). This sacrifice would not be necessary if young spores were being continuously cut off from the basal cell below. In an old sorus, therefore, one frequently finds germinated spores connected directly with the binu-

cleated cells at the base of the sorus by means of a granular aggregation of decomposition products (fig. 1, I) stretched out so that it simulates the expanded stalk of a spore of *Gymnosporangium*. At different levels on all sides, uninucleated spores are still present (pl. 2, C). Such a picture is misleading, for it suggests that all of the spores are at first sessile, but that by a stretching of the basal part, and as a result of lateral pressures, the spores are brought to different levels.

The spores developed at the margin of the sorus are greatly deformed and truncated (pl. 1, C). Though the terminal ones do germinate to form their internal basidia (fig. 1, H) they can be brought out into the open only by the great elongation of the spore below. Cross walls are not always laid down at right angles to the spore axis. This gives the spore cells the appearance of budding out at the side (fig. 1, G), especially if the wall of the spore above separates from the spore contents and undergoes mucilaginous disorganization.

Each cell of the internal promycelium has a single nucleus. One occasionally finds what might be taken to be two nuclei in each cell (fig. 1, M, N). One of the bodies, which is clearly a nucleus, is larger than the other. The smaller body seems to be more uniform in its composition, and stains less like a nucleus. Good fixation of these stages was not obtained. Binucleated sporidia are often found in the rusts, and it is possible that the original nucleus of the basidial cell sometimes divides precociously instead of waiting until it has passed up into the sporidium.

DISCUSSION

The amount of sterile tissue which is found overlying the sporogenous cells in a rust sorus may not now depend altogether on the depth at which the primordium develops, but it is generally conceded that the location of a sorus with respect to the host tissues did, in evolution, have a considerable influence in determining the type of primordium evolved. Arthur (2) and others early recognized the importance of this feature. In some forms of deep-seated aecidia, the primordium is composed of a mass of intertwined hyphae without orientation. The host tissues are crowded aside and, by subsequent disorganization of the fungus tissue at the center, a cavity is developed and food made available for the growth of spores. Where a resistant host tissue above is to be ruptured, one may find that the sorus primordium is composed of a palisade arrangement of hyphal cells thrust against the tissue to be broken away.

Had Lindfors (13) studied the organization of the telial sorus in *Gymnosporangium* and *Cronartium* and the uredinium in *Pucciniastrum* and *Cronartium*, or the deep-seated aecidia in certain *Peridermiums*, he would no doubt have anticipated the objections that have been raised against the theory that the sterile cells above the fusing cells are the morphological representatives of ancestral red-alga trichogynes, a theory which the propounder himself would no longer support. The nature of the sterile tissue lying above the fertile or fusing cells in a sorus is best understood, not by limiting one's studies of rusts to cell fusions, but by also investigating those sori where no fusions occur. Colley's method of study (4) of the white-pine blister rust, *Cronartium ribicola*, is to be commended particularly on this account.

As the writer interprets Colley's discussion of the development of the uredo sorus in *Cronartium ribicola*, the "peridium" is the exact homologue of the buffer tissue in Gymnosporangium. Certain cells of the uredo primordium become oriented vertically, and, by elongating, develop a thrust against the epidermis. These cells divide, and the upper daughter cells, in conjunction with their homologous neighbors, constitute the peridium made up of elongated cells. The subterminal cells now divide to give rise to spore initials above and basal cells below. The telial peridium is evidently developed in much the same way.

It has been found (5) that in several species of Gymnosporangium the teleutospores arise from the subterminal cells of the chains composing the primordium. The terminal cells, by swelling and elongating, serve as a buffer tissue to break open the overlying host tissues. The sacrifice of these terminal cells is made necessary by the fact that the tissues above the sorus are very tough, even in the case of leaf sori. The substance originally contained in the cells of the buffer tissue is not wasted, however. The teleutospore buds, by growing up through or between the sterile cells, absorb their pre-digested disorganization products, just as in all sorts of fungi one sees cases of self-parasitism in the form of "Durchwachsung" phenomena.

In the uredo sorus of *Pucciniastrum* (12, 6) the terminal cells of the sorus primordium are sterile and, though persisting as a "peridium," first lose their contents, swell and elongate, and function primarily as a buffer tissue. Adams (1), in a paper on the Peridermiums, shows that the fusing cells are merely intercalary cells in a space making buffer complex of parallel hyphae. In deep-seated sori there may be a half dozen or more cells above the fusing cells in the chain. The writer has found no evidence anywhere in the rusts that these chains are multicellular trichogynes.

Even though a buffer tissue persists more or less, as it does in *Gallowaya*, it should not be confused with a peridium. Morphologically, the latter structure in the rusts, if one is to take the peridium of the aecidium as the standard, is composed of spore and intercalary cells. In the uredo sorus of *Pucciniastrum* (6) the buffer tissue may be a true peridium, unless Kursanov (12) was correct in his statement as to this structure in *Pucciniastrum pirolae*. He claims that the intercalary cells which lie immediately below the terminal cells are cut off by the cells below. This would make the "peridium" of *Pucciniastrum* the homologue of the "peridium" of the uredinium of *Cronartium* and of the telium of Gymnosporangium, in neither of which is the structure a true peridium.

There has recently been described (8) a short-cycled strain of *Caecoma nitens* in which no spermogonia are developed. In every such case the aecidiospore arising without cell fusion is uninucleated, and on germination produces a two-celled promycelium with only two sporidia. The fact that *Gallowaya* develops at most only vestigial spermogonia does not seem to be of any particular effect on future growth processes. The internal promycelium is four-celled and produces at least four sporidia. Lindfors (13) says that in *Puccinia arenariae*, a short-cycled rust, the teleutospore produces a two-celled promycelium. Cell fusions do not occur in the sorus, the cells of the mycelium being already binucleated. A nuclear fusion in the spores is followed by two divisions, but the septa which divide the

structure into four cells are not laid down. There are two nuclei in each of the cells of the two-celled promycelium, and two nuclei are supposed to enter each sporidium. Lindfors shows two teleutospores germinating with long germ tubes, and he states that Grove had found four-celled promycelia in this species. Evidently this species of rust must receive further attention before it can be compared with the strain of the short-cycled *Caeoma nitens* which develops two-celled promycelia.

In any system of classification based on morphology it may be asked just how important, as showing relationship, is the manner of germination of a spore. Grove (11) says, "It is, indeed, doubtful whether the character upon which the Coleosporiaceae are united into one group, viz, the internal basidium, is really an indication of close affinity."

If the spores of *Chrysomyxa abietis*, which according to Kursanov (12) and Lindfors (13) are borne in chains, germinated with internal basidia instead of as they do, would this species be placed in the genus *Gallowaya* and in the group with *Coleosporium*? Except for the proliferation of the binucleated cell formed after cell fusion in this *Chrysomyxa*, there is not very much difference between it and *Gallowaya* so far as the organization of the sorus is concerned. In both cases the fusing cells are topped by two or three sterile cells. A short chain of binucleated cells is developed after cell fusion. These cells clearly never function as spores; they remain binucleated to the end, representing a short sporophytic generation. Nuclear fusion occurs in the outermost cells, so that a chain of uninucleated teleutospores is formed. Lindfors (13) doubts very much whether all of the uninucleated spores function in this *Chrysomyxa*, since in some cases the lower ones seem to lose most of their cytoplasm and become elongated. This is what often occurs in *Gallowaya*. In discussions of morphology it is not a question whether a structure functions, or whether a spore germinates, or what form the germ tube takes, but it should be asked, What are its phylogenetic antecedents?

Regardless of the merits of Grove's contention that *Ochropsora* is not one of the Coleosporiaceae, there can be no question that a system of classification based on the manner of spore germination is not a natural one. The writer (?) has shown that in certain strains of *Caeoma nitens* some of the aecidiospores produce long germ tubes which reinfect the host locally so that eventually teleutospores appear in the life cycle, while other aecidiospores, even from the same sorus, produce promycelia directly. So long as an unstable condition exists, such strains of orange-rust of *Rubus* can not be separated generically.

Galloway (10, p. 443) says: "The entire contents of the cell seem to be used up in the formation of the promycelium (sterigma) and the sporidium, and if this is not the case, the formation of secondary sporidia goes on until there is no protoplasm left." W. W. Diehl has informed the writer that he has seen cases where more than one sporidium was formed on the cell outgrowth in *Gallowaya*. Possibly in rare cases a true external promycelium is formed in *Gallowaya*. The writer has not seen young spores of this form with more than two nuclei. After germination, however, one can frequently find basidial cells with two bodies, as noted previously, both

very similar to nuclei and lying close together. If two sporidia are to be formed in succession from a single cell, one nucleus must remain behind after the first sporidium has been supplied. So far, this process has not been demonstrated for the rusts, although it is well known in the smuts. Even though two or more sporidia should be formed on the outgrowth from the same cell in *Gallowaya*, that outgrowth can not be called a promycelium, nor the cell from which it arises a teleutospore. Cells of teleutospores are mother cells, with which reduction divisions are normally associated. In this genus there is a series of binucleated "sporophytic" cells cut off from a fusion cell. Beginning with the oldest cell, the two nuclei fuse in regular order. The fusion nucleus in the oldest spore divides twice, a four-celled structure replacing the uninucleated cell. This may be sufficient evidence for assuming that the binucleated cells below are young spores and the uninucleated cells above them are certainly mature spores. Each four-celled structure is a protobasidium or internal promycelium.

In characterizing *Coleosporium*, Arthur (3), on the basis of what was then known of the telial sorus, says: "Teliospores sessile (by successive formation and displacement due to lateral pressure often appearing catenulate and pedicellate) * * *. By "successive formation" is evidently meant either that a new basal cell displaces the one from which a spore has been cut off, or the original basal cell buds out from the side to form a new spore. It is difficult to understand how the former process could take place once the primordium was organized. As far as the writer is aware, no one has yet reported a *Coleosporium* in which the basal cell buds out to form a number of spores. It is certain that such methods of spore formation do not occur in *Gallowaya*. The pedicellate appearance of its spores is in part due to displacement and lateral pressure, but its spores are nevertheless borne in chains. If the more or less persistent sterile tissue enveloping the uredo sori in *Hyalopsora*, *Pucciniastrum*, and *Cronartium* can be called a peridium, then the similar structure in *Gallowaya* must be referred to by the same designation.

SUMMARY

The mycelial hyphae of *Gallowaya pinicola* are composed of uninucleated cells. Aborted or vestigial spermogonia are sometimes developed between the mesophyll and the overlying hypodermal tissue. Spermatia are seldom formed.

The telial primordium first develops a buffer tissue composed of chains of cells, the terminal ones swelling or elongating somewhat, thus breaking open the overlying host tissues.

Cell fusions occur between intercalary cells in the chains composing the primordium. The fusing cells are the third or fourth cells from the ends.

Several binucleated cells are cut off above the fusion basal cell. These are at least potentially spores. Nuclear fusions occur in regular order, beginning with the oldest binucleated cell. As the terminal spore germinates, the lower end of its cell wall swells and elongates, thus thrusting the protobasidium still farther out in the sorus. Not all of the cells in a chain necessarily function as spores. Some of them become disorganized, owing to excessive elongation.

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SULPHATE CONTENT OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON¹

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INTRODUCTION

Previous investigations by the Office of Alkali and Drought-Resistant Plant Investigations and the Office of Biophysical Investigations of the Bureau of Plant Industry, United States Department of Agriculture, have shown that the Egyptian and Upland types of cotton are differentiated not merely by well-marked external botanical characters (6)² but by the physicochemical properties of their leaf-tissue fluids.

Since these physicochemical properties may be of significance as factors underlying differences in the drought and salt tolerance of the two forms as grown under the saline conditions of the Southwest, it has seemed desirable to investigate them in detail. In a first study (4) based on cultures made in 1920 and 1921 it was shown that Pima Egyptian and Meade and Acala Upland cottons are differentiated with respect to the osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration of their leaf-tissue fluids. These results have been confirmed by unpublished studies on these and on other varieties of Egyptian and Upland cotton grown in 1922, 1923, and 1924, and the investigations have been extended to the tissue fluids of the F₁ and F₂ hybrids. The results of these investigations are only partially published.

In their first discussion of the differentiation of the two types of cotton with respect to osmotic concentration as measured in terms of freezing-point depression, Δ , and specific electrical conductivity, κ , the writers pointed out that the higher value of the specific electrical conductivity and the possibly higher value of the ratio of specific electrical conductivity to freezing-point depression, κ/Δ , in the Egyptian type evidence a greater capacity of this type for the absorption and retention in solution of salts derived from the soil.

It has since been shown (5) that the chloride content of the leaf-tissue fluids of Pima Egyptian cotton is significantly higher than that of Meade, Acala, and Lone Star Upland cotton grown in immediate association. The present investigation has to do with the sulphate content of the Egyptian and Upland types of cotton.

¹ Received for publication Dec. 24, 1924; issued December, 1925.

² Reference is made by number (italic) to "Literature cited," p. 661.

MATERIALS AND METHODS

The experimental plants are in part the same as those considered in the earlier investigations. All were grown at the Cooperative Testing Station of the Bureau of Plant Industry in the Gila River Valley at Sacaton, Ariz. The study included:

A. A comparison of Pima Egyptian and Meade Upland cotton grown in 1922. Determinations were based on samples of tissue collected from individual plants.

B. A series of Pima Egyptian and Lone Star Upland plants grown in 1923. Determinations were based on samples taken from groups of plants. This is the series on which the chloride determinations for Pima and Lone Star cotton have been based (5).

Collection of samples and methods of preparation of tissue fluids are described elsewhere (4, 5). The sulphate content was determined (1) as follows:

Five to ten cubic centimeters of the expressed tissue fluid are placed in an 11.5 cm. porcelain evaporating dish, and 10 cubic centimeters of Benedict-Denis oxidizing reagent³ are added. This mixture is evaporated to approximate dryness on a water bath and then carefully ignited, at first over a small flame and finally to dull redness for a few minutes. After cooling, the ignition residue is dissolved in dilute hydrochloric acid, the solution filtered, and the sulphate precipitated from the filtrate by the addition of barium chloride and weighed as barium sulphate.⁴

Analyses were based on carefully pipetted samples of 5 to 10 cubic centimeters, preserved in sealed tubes after the addition of a drop of formaldehyde. Concentrations are expressed in terms of grams of sulphate (SO_4) per liter of tissue fluid.

RESULTS

The distribution of sulphate content in class units of 0.5 gm. of sulphate per liter in the single series of determinations made in the cultures of 1922, and in the two series of determinations made on the cultures of 1923, appears in Table I.⁵

³ This reagent is made by dissolving 25 gms. of crystalline copper nitrate, 25 gms. of sodium chloride, and 10 gms. of ammonium nitrate in enough water to make 100 cubic centimeters of solution. Ten cubic centimeters of the reagent are ample for 10 cubic centimeters of tissue fluid.

⁴ It is necessary to make "blank" determination of the reagent and to subtract this from the total weight of barium sulphate. By using high-grade chemicals, a blank of 0.0010 to 0.0035 gm. of barium sulphate is obtained.

⁵ In Table II of the paper presenting the results for chloride content in the 1923 cultures, the second series of determinations was divided into Part I and Part II. The sulphate analyses were made for samples taken from the south half of the plot only. Thus the sulphate determinations correspond to Part I only of the chloride series.

TABLE I.—Frequency distribution of sulphate content (in terms of grams of SO_4 per liter of fluid) in leaf-tissue fluids of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., in 1922 and 1923

Grams of SO_4 per liter	Cultures in 1922		Cultures in 1923			
	July 25 to Aug. 9		First series, July 29 to Aug. 14		Second series, Aug. 18 to Aug. 31	
	Pima	Meade	Pima	Lone Star	Pima	Lone Star
6.76 to 7.25=7	6					
7.26 to 7.75=7.50	2					
7.76 to 8.25=8	3					
8.26 to 8.75=8.50	2					
8.76 to 9.25=9		1				
9.26 to 9.75=9.50	3		2			
9.76 to 10.25=10	1	1				
10.26 to 10.75=10.50	2	4	2			
10.76 to 11.25=11	2	3	2			
11.26 to 11.75=11.50		1	8		2	
11.76 to 12.25=12		1	15		4	
12.26 to 12.75=12.50		1	12		3	
12.76 to 13.25=13		1	12	1	10	1
13.26 to 13.75=13.50	1	1	7		11	
13.76 to 14.25=14			1	2	15	2
14.26 to 14.75=14.50	2	4	2	5	5	1
14.76 to 15.25=15		1	2	13	7	1
15.26 to 15.75=15.50		3		6	6	7
15.76 to 16.25=16	2		1	9	1	12
16.26 to 16.75=16.50	1		1	4	2	13
16.76 to 17.25=17	1	1		14		7
17.26 to 17.75=17.50				4	1	1
17.76 to 18.25=18				4	1	8
18.26 to 18.75=18.50	1			3	1	4
18.76 to 19.25=19						2
19.26 to 19.75=19.50				1		4
19.76 to 20.25=20		1		1		1
20.26 to 20.75=20.50						
20.76 to 21.25=21		1			1	
21.26 to 21.75=21.50						1
21.76 to 22.25=22						1
22.26 to 22.75=22.50		2				
22.76 to 23.25=23		1				1
23.26 to 23.75=23.50		1				1
23.76 to 24.25=24						1
24.26 to 24.75=24.50						1
	29	29	67	67	70	70

These determinations show that while the sulphate contents of the two types of cotton are highly variable they are quite different, that of the Upland type (Meade and Lone Star) being apparently significantly higher than that of the Egyptian type (Pima).

The variability of the sulphate content is presumably due to the influence of substratum heterogeneity which has been shown to be a practically universal characteristic of experimental fields (2, 3). Illustrations of its influence on the tissue fluids of cotton are sufficiently developed in preceding papers (4, 5). Since the determinations on the two types were made in pairs, the true relationship between the concentrations may be shown best by a double-entry arrangement in tables in which the sulphate content of the two forms are shown on two axes. The form of table is identical with that employed in the writers' discussion of chloride content (5).

Table II presents the results for the first comparison of Pima and Meade plants (1922). Tables III and IV give the results for the first and second series of determinations on the Pima Egyptian and Lone Star Upland cultures of 1923.

TABLE IV.—Comparison of sulphate content of Pima Egyptian and Lone Star Upland cotton grown under irrigation at Sacaton, Ariz., 1923.
Second series of determinations

Total.	2	4	3	10	11	15	5	7	6	1	2	17.00	17.50	18.00	18.50	19.00	19.50	20.00	20.50	21.00	Total
24.50									1	1											1
24.00																					1
23.50									1												1
23.00								1													1
22.50																					1
22.00																					
21.50							1		1												1
21.00																					1
20.50																					
20.00						1	1		1												
19.50							1		1		1										1
19.00				1					1												4
18.50				2					1												2
18.00		1	1	1	1			1	1											1	4
17.50						1	1														1
17.00				2	1	4															7
16.50		1		2	4	2	2	2													13
16.00			1	1	4	3	1	2													12
15.50	2		1	1	1	2															7
15.00							1	1													1
14.50		1																			1
14.00		1				1															1
13.50																					2
13.00																					
12.50																					
12.00																					
11.50																					
	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	17.00	17.50	18.00	18.50	19.00	19.50	20.00	20.50	21.00	Total

LONE STAR

PIMA

Except for a few outlying analyses,⁶ the sulphate content of the Upland type is higher than that of the associated Egyptian plants. This is shown by the fact that practically without exception the sulphate contents of the Upland variety lie above the diagonal cells (marked by the short rules) about which they should be concentrated if the content were the same in the two types.

Determining the coefficients of correlation between the sulphate content of associated Egyptian and Upland plants, the values are set forth in Table V.

TABLE V.—Correlation between sulphate content of associated plants or groups of plants of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., in 1922 and 1923

	Table	Correlation coefficient and probable error, $r \pm E_r$	Ratio of correlation to probable error, r/E_r
Comparison of Pima Egyptian and Meade Upland cotton, 1922: First series, July 25 to Aug. 9, correlation between Pima and Meade.	II	0.6521±0.0720	9.06
First comparison of Pima Egyptian and Lone Star Upland cotton, 1923: First series, July 29 to Aug. 14. Correlation between Pima and Lone Star	III	.1047±.0815	1.28
Second comparison of Pima Egyptian and Lone Star Upland cotton, 1923: Second series, Aug. 18 to Aug. 31. Correlation between Pima and Lone Star	IV	.3970±.0679	5.85

The three coefficients are all positive, and two of them may be considered statistically significant in comparison with their probable errors. They are, however, more irregular in actual magnitude than those demonstrated for chloride content (5).

The positive correlation indicates that the sulphate contents of associated Egyptian and Upland plants are influenced in a similar manner by some common extrinsic factor. It also indicates the necessity for considering the correlation between associated determinations in the calculation of the probable error of the difference between them.

The mean sulphate contents of the individual series and their probable errors, the differences in the mean sulphate content of the Egyptian and Upland types, and the probable errors of these differences appear in Table VI. The probable errors of the differences are calculated with due regard to the correlations between the variables by the formula

$$\sigma^2_{(p-u)} = \sigma^2_p + \sigma^2_u - 2r_{pu} \sigma_p \sigma_u$$

The differences are from 9 to 26 times as large as their probable errors, and hence are clearly significant. Absolute differences are, on the average, 3 to 4 grams of sulphate per liter. Percentage differences, calculated by using the sulphate content of the Upland type as a base, range from about 18 to about 28 per cent.

⁶ These exceptions are presumably due to experimental errors but are included in order to avoid any possibility of the selection of data in the formulation of the conclusion.

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TABLE VI.—Mean sulphate content (in terms of grams of SO_4 per liter) of tissue fluids of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., 1922 and 1923

	N	Mean sulphate content for Egyptian cotton	Mean sulphate content for Upland cotton	Difference between Egyptian and Upland cotton		
				Absolute difference and probable error	Ratio of absolute difference to probable error	Percentage difference
Comparison of Pima Egyptian and Meade Upland cotton, 1922: First series, July 25 to Aug. 9. Comparison of Pima and Meade.	29	10.5517±0.4447	14.6724±0.5385	-4.1206±0.4189	9.84	28.08
First comparison of Pima Egyptian and Lone Star Upland cotton, 1923: First series, July 29 to Aug. 14. Comparison of Pima and Lone Star.	67	12.5373±.1022	16.2164±.0801	-3.6791±.1410	26.10	22.69
Second comparison of Pima Egyptian and Lone Star Upland cotton, 1923: Second series Aug. 18 to Aug. 31. Comparison of Pima and Lone Star.	70	14.1642±.1294	17.3357±.1793	-3.1714±.1750	18.12	18.29

SUMMARY AND CONCLUSION

The sulphate content of the leaf-tissue fluids of Meade and Lone Star Upland cotton has been investigated in comparison with that of Pima Egyptian cotton as grown under irrigation in the Gila River Valley, Sacaton, Ariz.

The sulphate content of the Upland varieties (Meade and Lone Star) is higher than that of the Egyptian variety (Pima). The differences are clearly significant in comparison with their probable errors and range from 3 to 4 grams per liter, or from 18 to 28 per cent of the Upland value.

These results as compared with those of the earlier study of chloride content show that, as far as the varieties investigated are concerned, the behavior of these two types of cotton with respect to the absorption of chlorides and sulphates is quite different, the Egyptian type taking up larger quantities of chlorides, and the Upland types absorbing larger quantities of sulphates.

The relationship between the concentration of these ions in the plant, the relation of their concentration in the plant to that of their concentration in the soil solution, and their behavior in inheritance are under investigation.

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ABORTION-BACTERIN TREATMENT OF COWS HAVING UDDERS INFECTED BY *BACTERIUM ABORTUS*¹

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INTRODUCTION

For a number of years investigators of bovine infectious abortion have recognized that when cows become infected with *Bacterium abortus* the microorganism shows a tendency to localize in the udder and to live and multiply in this organ for widely varying periods of time. It is also common knowledge that *Bact. abortus* readily thrives in the uteri of pregnant bovines, but is seldom found in this organ longer than two months after the expulsion of a fetus, living or dead. Schroeder and Cotton,² in their efforts to determine whether the organism becomes localized in the cow's body elsewhere than in the organs mentioned, found the infection present in the supramammary lymph glands and certain other lymph glands at the brim of the pelvis. The present writers were able in a single instance to isolate the infection from the femoro-tibial joint of a cow; lameness in this joint prompted the search for the organism in this particular location.

Although in rare instances the infection has been found localized in organs other than the uterus, udder, and associated lymph glands of the latter, it seems highly probable that it is commonly the udder which harbors the infection in the animal between gestation periods, and acts as a source of infection for the uterus when conditions are favorable for the multiplication of *Bact. abortus* therein. When second and third abortions in the same animal are observed as being associated with *Bact. abortus*, and placental infection with this organism in those cases which harbor the infection is common, even though a living calf is produced, it seems that the devising of a method whereby this focus of infection may be eliminated would accomplish much toward solving the abortion problem. Some investigators have been of the opinion that success in such an undertaking may not be unattainable. Experimental use has been made of certain drugs which, it was thought, might possibly exert antiseptic action during their elimination through the udder. Results from such medication seem to have yielded but slight encouragement so far.

In this paper are discussed the procedure followed and the results obtained in an endeavor to overcome *Bact. abortus* udder infection by repeated subcutaneous administrations of *Bact. abortus* bacterin. In undertaking this line of treatment it did not appear wholly unreasonable to infer that any immunity which might be engendered by such treatment would have a desirable effect at the seat or seats of the infection.

¹ Received for publication Nov. 21, 1924; issued December, 1925.

² SCHROEDER, E. C., and COTTON, W. E. SOME FACTS ABOUT ABORTION DISEASE. Jour. Amer. Vet. Med. Assoc. (n. s. 3) 50: 321-330. 1916.

EXPERIMENTAL PROCEDURE

The experimental work was carried out in a herd of approximately 200 head of dairy cattle in which infectious abortion had prevailed for about five years. An agglutination test of the herd, made March 17, 1922, showed that the blood serum of 22 cows caused clumping of a *Bact. abortus* suspension in amounts as small as 0.001 c. c., and that the end point in the case of 8 others was 0.002 c. c. Results of this test on these 30 cows are given in Table I.

TABLE I.—Blood-serum reactions obtained in *Bacterium abortus* agglutination tests on 30 cows

Cow No.	Quantity of blood serum (cubic centimeters)						Cow No.	Quantity of blood serum (cubic centimeters)					
	0.04	0.02	0.01	0.005	0.002	0.001		0.04	0.02	0.01	0.005	0.002	0.001
17.....	+	+	+	P	P	S	432.....	+	+	+	+	+	+
49.....	+	+	+	+	+	+	434.....	+	+	+	+	+	+
51.....	+	+	+	+	S	S	435.....	+	+	+	+	+	P
54.....	+	+	+	+	S	S	439.....	+	+	+	+	+	+
67.....	+	+	+	+	+	+	443.....	+	+	+	+	+	+
71.....	+	+	+	+	+	P	445.....	+	+	+	+	+	+
84.....	+	+	+	+	P	S	465.....	P	P	P	P	P	P
89.....	+	+	+	+	+	P	50.....	+	+	+	+	+	—
223.....	+	+	+	+	+	+	247.....	+	+	+	+	+	—
245.....	+	+	+	+	+	+	421.....	+	P	S	S	S	—
254.....	+	+	+	+	+	+	441.....	+	+	+	+	+	—
409.....	+	+	+	+	+	+	443.....	+	+	P	P	S	—
417.....	+	P	P	P	S	S	448.....	+	P	S	S	S	—
418.....	+	+	+	+	+	+	466.....	P	P	P	P	P	—
431.....	+	+	+	+	+	+	468.....	S	S	S	S	S	—

+ = Pronounced agglutination.
P = Partial agglutination.

S = Slight agglutination.
— = No agglutination.

In making these agglutination tests six different quantities of blood or milk serum were used. There is placed in six different test tubes these respective amounts: 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 c. c. There is then added to each tube 1 c. c. of a 0.5 per cent carbolized suspension of *Bact. abortus* similar in density to a barium-sulphate suspension resulting from the addition of 1 c. c. of a 1:100 solution of barium chloride to 99 c. c. of a 1:100 solution of sulphuric acid. Results are indicated by these signs: +, which signifies pronounced agglutination of the bacteria; P, clumping incomplete; S, a trace of agglutination; and —, no agglutination. Readings of the tests were made after incubation for 36 hours.

After identifying the marked reactors to the abortion test, composite samples each containing 40 to 50 c. c. of milk or udder secretions, were taken from these 30 animals, as were blood specimens also, with the idea of detecting those cows which carried the infection in their udders.

The milk was kept in the refrigerator over night, after which the cream layer was pipetted off. The specimens were then thoroughly shaken, warmed to body temperature, and injected intra-abdominally into guinea pigs in 5 c. c. amounts. Two guinea pigs were used in most cases for making this preliminary test; in two or three cases four were inoculated. In obtaining the agglutinin titer of the milk, a small quantity of rennet was used to curdle the specimens. Milk

serum was used because it was more satisfactory to read the agglutination tests from it than from the milk.

It was suspected that success or failure in infecting the guinea pigs might depend to some extent upon the quantity of milk the cows gave at the time the milk specimens were taken, or, in other words, upon the concentration of the bacteria; so a record was kept of the quantity of milk produced by each animal during the 24-hour period previous to the collection of the various samples.

A practice was made of killing the inoculated guinea pigs in from five to eight weeks after the intra-abdominal injections. Serum-agar slants in nearly every case were sown with spleen tissue as a means of detecting *Bact. abortus* infection, and the cultures were subjected to incubation at 37° C. in closed jars in which 10 per cent of atmosphere was displaced by carbon-dioxide gas.

Table II shows the quantity of milk produced by each of the 30 cows during the 24-hour period just prior to the collection of the samples, the agglutination results with blood and milk sera, and outlines the procedure followed in inoculating, autopsying, and culturing the guinea pigs.

The results obtained from the milk inoculations of guinea pigs proved that 17 of the 30 cows carried *Bact. abortus* in their udders. It is probable that the number of carriers was considerably larger than was indicated by the inoculation results. The number of guinea pigs used for each case was small, and death from enteritis, pneumonia, etc., was of frequent occurrence—factors which may have rendered the results somewhat misleading.

Sixteen of the udder-infected cows were used for testing the value of the abortion-bacterin treatment. This number was divided equally into two groups. In order to make the division as nearly uniform as possible, an effort was made to include in each group animals which had been carriers for similar lengths of time as based on their histories and records of previous serological tests.

The eight cows of one group (Nos. 51, 247, 409, 432, 435, 439, 442, and 468) were each given a subcutaneous injection of 10 c. c. of abortion bacterin on the following dates: May 24, May 29, June 3, June 9, June 15, and June 21 (1922). The bacterin was prepared from numerous strains of *Bact. abortus* which had been under artificial cultivation for a year or longer. The organisms were killed by placing the flasks containing the suspensions in a water bath maintained at a temperature of 60° C. for one-half hour. The bacterin consisted of approximately one billion organisms per cubic centimeter. The eight animals of the second group were used as controls.

In order to determine whether there was any systematic disturbance resulting from such bacterin injections which might prove detrimental to the animals from the standpoint of production or otherwise, temperatures of the treated cows were taken on the days that the injections were made and three times daily on the four following days. Their daily milk yield was also recorded. There was usually a slight reduction in milk-yield on the day following the treatment and sometimes on the second day. A slight rise of temperature was observed in a few of the cows following the injections. In some of the animals there was as welling at the point of inoculation that did not entirely disappear for a week or ten days.

TABLE II.—Data of agglutination and inoculation tests of blood and milk samples from the 30 cows tabulated in Table I

Cow No.	Milk yield in pounds ^a	Agglutination results ^b		Guinea-pig inoculations ^c			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
17	9.8	+	P P P S —	74799	—	May 7	+ P P P P —	Spleen much enlarged	Positive.
49	Dry.	+	+	74900	—	do.	+ P P S S —	No visible lesions	Do.
51	47	+	+	74777	Apr. 8	—	(^d)	do.	Not cultured.
54	26.5	+	+	74370	Apr. 18	—	+ P P P P —	Spleen enlarged	Negative.
67	Dry.	+	+	74901	—	May 7	+ + + + + P P	No visible lesions	Positive.
71	42.7	+	+	74802	—	do.	+ + + + + P P	do.	Do.
84	Dry.	+	+	74795	—	do.	—	No Bact. abortus lesions	Negative.
89	7.5	+	+	74796	—	do.	(^d)	Spleen enlarged	Do.
223	Dry.	+	+	74787	Apr. 12	—	+ + + + + P P	No Bact. abortus lesions	Not cultured.
245	44.5	+	+	74788	—	May 7	+ P P P P P	Spleen enlarged	Positive.
254	30	+	+	74905	—	do.	+ P P P P P	No Bact. abortus lesions	Do.
409	34.4	+	+	74341	Apr. 11	—	(^d)	do.	Negative.
417	17.6	+	+	74342	—	May 7	+ + + P P —	No visible lesions	Positive.
418	33.2	+	+	74783	Apr. 20	—	(^d)	Spleen much enlarged	Do.
431	17.6	+	+	74784	Apr. 19	—	+ P P P P —	No Bact. abortus lesions	Not cultured.
432	24.4	+	+	73105	Apr. 1	—	P P P P S —	Spleen enlarged and nodular	Positive.
		+	+	74912	Apr. 2	—	(^d)	No visible lesions	Negative.
		+	+	74772	Apr. 2	—	—	No Bact. abortus lesions	Not cultured.
		+	+	74773	—	May 7	+ + + P P —	Spleen enlarged	Negative.
		+	+	74791	Apr. 10	—	(^d)	No Bact. abortus lesions	Positive.
		+	+	74764	—	May 7	P P S S —	Spleen slightly enlarged	Negative.
		+	+	74765	Apr. 26	—	(^d)	No Bact. abortus lesions	Positive.
		+	+	74602	—	May 1	—	do.	Not cultured.
		+	+	74603	—	do.	—	do.	Negative.
		+	+	74785	—	May 7	—	No visible lesions	Do.
		+	+	74786	Apr. 13	—	(^d)	No Bact. abortus lesions	Do.
		+	+	74781	Apr. 7	—	(^d)	do.	Do.
		+	+	74782	Apr. 1	—	(^d)	do.	Do.
		+	+	74910	Apr. 20	—	(^d)	do.	Negative.
		+	+	74911	May 7	—	—	do.	Do.
		+	+	74739	—	May 7	—	No visible lesions	Do.
		+	+	74740	—	do.	—	do.	Do.
		+	+	74770	—	do.	—	do.	Do.
		+	+	74771	—	do.	+ P P —	do.	Positive.

	20.1	34.6	17.7	34	23.4	21.8	18.3	49.3	7.9	28.6	25.7	9.8	24.7	23.1	
424	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	P P P P P P	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	P P P P P P	S S S S S S	Not cultured.
435	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	++ ++ ++ ++ ++	Negative.
															Positive.
439															Negative.
442															Positive.
445															Negative.
465															Do.
															Positive.
															Do.
															Negative.
															Do.
50															Positive.
247															Do.
421															Negative.
441															Do.
443															Positive.
448															Negative.
466															Do.
468															Positive.
															Negative.
															Positive.
															Do.

* For the 24 hours (Mar. 28, 1922) just prior to taking samples.

* In this and following tables the quantities of blood or milk sera used in the agglutination tests were 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 c. c., respectively For explanation of symbols, see footnote to Table I.

* All inoculated March 29, 5 c. c. milk per pig.

* No test.

* "No *Bact. abortus* lesions" signifies no perceptible enlargement or nodular condition of spleen, absence of visible necrotic foci in liver, absence of visible lesions involving testicles, epididymal, bones, articulations, etc.

Table III shows the temperature reactions and variations in milk yield that followed the initial treatment. They are more or less typical of the temperature and milk yield variations that occurred following the subsequent bacterin injections.

TABLE III.—Temperatures and milk yield of cows for four days following first abortion-bacterin treatment on May 24, 1922

Cow No.	Date	8 a. m.	12 m.	4.30 p. m.	Milk yield	Cow No.	Date	8 a. m.	12 m.	4.30 p. m.	Milk yield
		° F.	° F.	° F.	Pounds			° F.	° F.	° F.	Pounds
51	May 24..		101.0	101.1	46.7	435	May 24..			101.0	29.1
	May 25..	103.7	103.2	103.0	43.3		May 25..	101.8	101.6	101.4	25.9
	May 26..	103.2	101.6	101.8	45.3		May 26..	101.6	101.8	101.7	29.8
	May 27..	101.8	101.7	102.0	44.6		May 27..	101.5	101.4	101.8	29.5
	May 28..	101.9	102.0	102.0	47.3		May 28..	101.2	101.2	101.0	28.2
247	May 24..			100.6	52.5	439	May 24..		100.8	100.8	16.8
	May 25..	103.4	103.0	101.4	49.7		May 25..	101.5	101.8	101.5	17.7
	May 26..	101.0	101.4	101.5	50.9		May 26..	101.6	101.5	101.6	16.3
	May 27..	101.2	101.2	100.6	52.2		May 27..	101.7	101.7	101.8	18.6
	May 28..	101.0	101.2	101.0	51.0		May 28..	101.6	101.7	101.8	17.5
442	May 24..			101.2	23.9	409	May 24..			101.0	32.9
	May 25..	101.8	101.6	101.4	22.6		May 25..	100.8	100.9	101.0	31.0
	May 26..	101.0	101.3	101.2	23.2		May 26..	100.6	100.9	101.3	33.1
	May 27..	101.2	101.2	101.4	23.2		May 27..	101.2	101.2	101.0	31.6
	May 28..	101.5	101.4	101.3	22.8		May 28..	101.3	101.4	101.0	31.1
432	May 24..			101.2	22.0	468	May 24..		100.4	100.3	21.4
	May 25..	102.0	101.8	101.5	19.3		May 25..	100.8	101.3	101.4	22.2
	May 26..	101.2	101.2	101.5	19.4		May 26..	101.9	101.7	101.8	20.3
	May 27..	101.1	101.2	101.4	21.5		May 27..	101.8	102.0	102.0	20.9
	May 28..	101.5	101.4	101.0	20.8		May 28..	101.8	102.0	101.9	22.2

Composite samples of milk were obtained from animals of the treated and control groups on six different dates following the administration of the bacterin, with the object of determining whether the bacterin injections had exerted curative action. Blood samples were also usually taken on the same dates. The blood and milk samples collected on the various dates were handled in a manner similar to that described for the original collection. The periods that intervened between the completion of the bacterin treatments and the collection of the different samples were approximately 2, 4, 6, 11, 15, and 20 months.

Tables IV to XII, inclusive, show the manner in which the blood and milk samples reacted to the agglutination test, results of the milk inoculations of guinea pigs, the serological and cultural results obtained from the inoculated guinea pigs, and other data.

DISCUSSION OF RESULTS

In the writers' studies of the results of numerous guinea-pig inoculations it was found that the nature of the agglutination results of the blood serum, five to eight weeks after inoculation, was in most cases a reliable index to the presence or absence of *Bacterium abortus* infection, and that to depend wholly upon macroscopic lesions as indicators of such infections may in many cases prove misleading. This was clearly evidenced by the fact that it was frequently possible to isolate from 150 to 200 colonies of the abortion organism from spleens of inoculated guinea pigs which were normal in their gross arance. In only a few instances did reacting guinea pigs give ive cultural results.

In the first guinea-pig inoculation test following the bacterin treatment of the cows it was demonstrated that 7 treated and 5 control cows were eliminating the abortion organism in their milk; and in the second series of inoculations, 4 treated and 6 control cows showed infected udders. Before the third test was made one of the treated cows (No. 51) died. The third test indicated that 4 treated and 4 control cows were carriers of the microorganism. Five treated and 5 control cows produced milk that infected guinea pigs on the fourth test; 3 treated cows and 5 controls gave milk from which guinea pigs were infected on the fifth test; and 3 treated cows and 5 controls gave milk from which guinea pigs were infected on the sixth test.

The inoculation results indicated that the guinea-pig method of detecting *Bact. abortus* infection is by no means ideal or perfect. Not only was there a lack of uniformity in the results of the inoculation tests of milk of certain cows collected on different dates—irregularities which may have been due to an intermittent appearance of the infection in the milk—but the same sample of milk when administered to several guinea pigs in the same quantities frequently yielded conflicting results. The guinea-pig cultural results recorded in Table X plainly show that volumetrically equal doses of material containing *Bact. abortus* do not always affect the animals in a similar manner.

The number of bacterin-treated cows which continued to be carriers of *Bact. abortus* 608 days after the immunizing treatment, as indicated by the inoculation results, was 3, or approximately 43 per cent, while the number of controls was 5, or 62½ per cent. Although the number of carriers after the treatment was larger in the control group, the difference did not appear to be sufficiently marked to justify the conclusion that the bacterin injections had any appreciable effect in overcoming the udder infection. Table XI shows the agglutination reactions that were obtained with blood serum from the 16 cows obtained on the different dates, and Table XII shows the agglutination reactions with the milk whey from the same animals, as well as the milk yield for a 24-hour period preceding the collection of the samples. The blood reactions, while showing some variation in intensity on the different dates of collection, are more uniform, possibly, than might be expected when it is considered that a number of the cows, including Nos. 432, 17, 84, and 441, ceased, as far as the guinea-pig tests showed, to carry the infection in their udders for a period of from one to one and a half years.

During the experiment comparatively few abortions occurred, while barrenness troubles were rather pronounced. The 15 cows which were carried through the experiment, continuing approximately two years, have produced 16 living calves. Five cows are now (April 3, 1924) heavy with calf. Cow 439 aborted a fetus in which *Bact. abortus* was present. Cow 84 aborted twice, the presence of *Bact. abortus* being demonstrated in the first abortion, but failure was experienced in isolating the organism in the second. Two of the cows (465 and 468) became pregnant only after ten services. Cow 247 seems to be permanently barren. Several other cows were bred five to six times before conception occurred. Table XIII gives some breeding data relative to the group since the experiment was undertaken.

TABLE IV.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 63 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds*	Agglutination results		Guinea-pig inoculations *			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
51	18.2	+++ + + S -	+ + P S - -	76089		Nov. 2	- - - - -	No visible lesions	Negative.
247	-----	+++ + + + + +	+ + P S - -	76090		do	- - - - -	do	Do.
				76091		do	- - - - -	do	Do.
				76046		do	- - - - -	do	Do.
				76047		do	- - - - -	do	Do.
409	25	P P P + + +	+ + + + - -	76048		do	+ + + + S -	Spleen enlarged	Positive.
				76049		do	P S S - -	Spleen slightly enlarged	Do.
				76041		do	- - - - -	No visible lesions	Do.
				76042		do	- - - - -	do	Negative.
432	18.6	+++ + + + + +	+ + P P - -	76095		do	P P P S - -	Spleen slightly enlarged	Positive.
				76096		do	+ P P P S -	No visible lesions	Do.
				76097		do	- - - - -	do	Negative.
				76092		do	+ + + + P S	Spleen enlarged; liver lesions	Positive.
435	27.5	S S S S P P	+ S S - - -	76093		do	- - - - -	No visible lesions	Negative.
439	14	P P + + + +	+ + + + P S	76094		do	P - - - -	Liver lesions; spleen normal	Positive.
				76086		do	+ + + + + P	No visible lesions	Do.
				76087		do	- - - - -	do	Negative.
				76088		do	- - - - -	do	Do.
442	17.4	P P P P + +	+ P P S - -	76043		do	- - - - -	do	Do.
				76044		do	S S S - -	do	Positive.
				76045		do	+ P P S - -	Spleen enlarged; necrotic foci in liver	Do
				76080		do	P S - - -	Spleen nodular; liver lesions	Do
468	17.2	P P P + + S	+ + P S - -	76081		do	- - - - -	No visible lesions	Negative.
				76082		do	P P P - -	Spleen and liver lesions	Positive.

CONTROLS

17	18.3	P P S S S S	+ P P P S -	76083		Nov. 2	- - - - -	- - - - -	No visible lesions	Negative.
				76084		do.	- - - - -	- - - - -	do.	Do.
71	18.3	P P + + + +	+ P P P S -	76085		do.	- - - - -	- - - - -	Spleen nodular; liver lesions	Positive.
				76518		do.	- - - - -	- - - - -	Liver lesions; spleen normal	Do.
				76519		do.	- - - - -	- - - - -	Spleen enlarged; liver lesions	Do.
84	24	+ + P P P P	+ + P S -	76520		do.	- - - - -	- - - - -	No visible lesions	Negative.
				76527		do.	- - - - -	- - - - -	do.	Do.
				76528		do.	- - - - -	- - - - -	do.	Do.
223	51	P + + + + +	+ + + S -	76529		do.	- - - - -	- - - - -	do.	Do.
				76524		do.	- - - - -	- - - - -	Spleen and liver lesions	Positive.
				76525		do.	- - - - -	- - - - -	No visible lesions	Negative.
254	19.6	+ + + + P P	+ + + + P -	76526		do.	- - - - -	- - - - -	Spleen enlarged	Positive.
				76530		do.	- - - - -	- - - - -	No visible lesions	Do.
				76531		do.	- - - - -	- - - - -	do.	Do.
441	19.5	+ + + + P P	P P P - - -	76532		do.	- - - - -	- - - - -	do.	Negative.
				76533		do.	- - - - -	- - - - -	do.	Do.
				76534		do.	- - - - -	- - - - -	do.	Do.
				76535		do.	- - - - -	- - - - -	No autopsy	Do.
465	19.6	P + + + P -	+ + S - - -	76521	Sept. 13	do.	- - - - -	- - - - -	Spleen and liver lesions	Not cultured.
				76522		Nov. 2	- - - - -	- - - - -	No visible lesions	Positive.
				76523		do.	- - - - -	- - - - -	Spleen and liver lesions	Negative.
466	Dry.	+ P P P P P	+ + + + + +	76536		do.	- - - - -	- - - - -	Spleen enlarged	Positive.
				76537		do.	- - - - -	- - - - -	Spleen and liver lesions	Do.
				76538		do.	- - - - -	- - - - -	do.	Do.

^a For the 24 hours (Aug. 23, 1922) just prior to taking sample.
^b All inoculated Aug. 24, 1922, 8 c. c. milk per pig.
^c No test.

TABLE V.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 110 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds ^a	Agglutination results		Guinea-pig inoculations ^b			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
				Guinea pig No.	Date of death (1922)				
		Blood	Milk		Died	Killed			
51	21.6	+	+	+	+	P	76962 76963 76964 76977 76978 76979 76953 76954 76955 76971 76972 76973 76959 76960 76961 76968 76969 76970 76950 76951 76952 76938 76939 76940	Nov. 24 do. do. Oct. 23 Nov. 24 do. Nov. 17 Nov. 24 do. Oct. 17 Nov. 24 do. do. do. do. do. do. do. do. do. Nov. 2	— —

CONTROLS

[illegible]

^c For the 24 hours (Oct. 9, 1922) just prior to taking samples.

^b All inoculated (Oct. 10, 1922) 5 c. c. milk per pig.

^c No test.

TABLE VI.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 167 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds*	Agglutination results		Guinea-pig inoculations ^b			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
		Blood	Milk	Guinea pig No.	Date of death (1922)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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247	21.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

CONTROLS

[illegible]

• For the 24 hours (Dec. 5, 1922) just prior to taking samples.

* All inoculated Dec. 6, 1922, 5 c. c. milk per pig.

c No test.

TABLE VII.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 312 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds	Agglutination results		Guinea-pig inoculations ¹		Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea-pig No.	Date of death (1923) Died Killed			
247	18	+	+	79980	June 25	+ P S	No visible lesions	Positive.
		+	+	79981	do	+ + +	do	Do.
		+	+	79982	do	+ + +	do	Do.
		+	+	79983	do	P S	Spleen enlarged	Do.
432	21	+	P	79992	do	P P	No Bact. abortus lesions	Negative.
		+	P	79993	do	P P	do	Do.
		+	P	79994	May 26	(^c)	do	Do.
		+	P	79995	June 25	—	do	Do.
435	15.9	+	+	79996	do	—	No visible lesions	Do.
		+	+	79997	do	—	do	Do.
		+	+	79998	do	—	do	Do.
		+	+	79999	do	—	do	Do.
439	11.2	P	P	80100	do	P S	do	Do.
		P	P	80101	do	—	do	Do.
		P	P	80102	do	—	do	Do.
		P	P	80103	do	—	do	Do.
409	37.3	+	+	79988	do	+ + +	do	Positive.
		+	+	79989	do	+ + +	do	Negative.
		+	+	79990	do	—	do	Do.
		+	+	79991	do	—	do	Do.
442	33	P	P	80108	May 26	(^c)	No Bact. abortus lesions	Positive.
		P	P	80109	do	—	do	Not cultured.
		P	P	80110	June 25	—	do	Negative.
		P	P	80111	do	—	do	Positive.
468	9.9	+	+	80120	do	—	No visible lesion	Do.
		+	+	80121	do	—	do	Do.
		+	+	80122	May 13	—	No Bact. abortus lesions	Negative.
		+	+	80123	June 25	P S	do	Positive.

CONTROLS

[illegible]

^a For the 24 hours (May 8, 1923) just prior to taking samples.

^b All inoculated May 9, 1923, 5 c. c. milk per pig.

^cNo test.

TABLE VIII.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Milk samples collected 453 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield, in pounds ^a	Agglutination results, milk	Guinea-pig inoculations ^b			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
			Guinea-pig No.	Date of death (1923)				
				Died	Killed			
247	19.8	+ + + P S —	81683 81684 81685	Oct. 5 — —	Nov. 7 do —	(c) + + + S — + + + P S —	No Bact. abortus lesions No visible lesions do	Not cultured. Positive. Do.
432	8.2	+ + P P —	81689 81690 81691	— — —	do do do	— — —	do do do	Negative. Do. Do.
435	20	+ + S — —	81692 81693 81694	— — —	do do do	— — —	do do do	Do. Do. Do.
439	2.2	+ + + + +	81695 81696 81697	— — —	do do do	+ S — — —	do do do	Positive. Negative. Do.
409	24.6	+ + S S —	81698 81699 81900	— — —	do do do	— — —	do do do	Do. Do. Do.
442	28.1	+ + P S —	81686 81687 81688	— — —	do do do	— — —	do do do	Do. Do. Do.
468	3.6	+ + + P S —	81901 81902 81903	Oct. 27 — —	Nov. 7 do do	(c) + + S — —	No Bact. abortus lesions No visible lesions do	Not cultured Positive. Negative. Positive.

CONTROLS

17	Dry.	+	+	+	P	S	-	81904					No visible lesions	Negative.
								81905	-	-	-	-	do	Do.
71	24.8	+	+	+	P	P	P	81906	-	-	-	-	do	Do.
								81907	+	+	+	+	Spleen nodular	Positive.
								81908	+	+	+	+	No visible lesions	Do.
84	1	P	P	P	P	S	-	81909	+	+	+	+	Spleen nodular	Do.
								81910	-	-	-	-	No visible lesions	Negative.
								81911	-	-	-	-	do	Do.
223	10	P	P	S	-	-	-	81912	-	-	-	-	do	Do.
								81913	-	-	-	-	do	Do.
								81914	-	-	-	-	do	Do.
254	34.8	+	+	+	P	P	-	81915	-	-	-	-	do	Positive.
								81916	+	+	+	+	Spleen and liver lesions	Negative.
441	Dry.	+	+	+	+	+	+	81917	+	+	+	+	No visible lesions	Positive.
								81918	-	-	-	-	do	Do.
								81919	-	-	-	-	do	Negative.
								81920	-	-	-	-	do	Do.
465	14.4	+	+	+	P	-	-	81921	-	-	-	-	do	Do.
								81922	-	-	-	-	do	Do.
								81923	-	-	-	-	do	Do.
466	Dry.	+	+	+	P	P	S	81924	+	S	-	-	do	Do.
								81925	+	+	+	+	Spleen nodular	Positive.
								81926	+	+	S	-	No visible lesions	Do.
								81927	+	+	+	+	Spleen nodular	Do.

^a For the 24 hours (Sept. 26, 1923) just prior to taking samples.
^b All inoculated Sept. 27, 1923, 5 c. milk per pig.
^c No test.

CEREAL INVESTIGATIONS.
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TABLE IX.—Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Milk samples collected 608 days after bacterin treatment

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds *	Agglutination results, milk	Guinea-pig inoculations *			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
			Guinea pig No.	Date of death (1924)				
				Died	Killed			
247	16.1	+ + + + - -	83703	- - - - -	Mar. 29	+ + + + +	No visible lesions.	Positive.
			83704	- - - - -	do.	- - - - -	do.	Negative
			83705	- - - - -	do.	- - - - -	do.	Do.
			83706	- - - - -	do.	- - - - -	do.	Do.
432	Dry.	+ + P P P P	83466	Mar. 7	- - - - -	(c)	No Bact. abortus lesions	Do.
			83467	- - - - -	Mar. 29	- - - - -	No visible lesions.	Do.
			83468	- - - - -	do.	- - - - -	do.	Do.
			83469	- - - - -	do.	- - - - -	do.	Do.
435	10.6	P P P - - -	83497	- - - - -	do.	- - - - -	do.	Do.
			83498	- - - - -	do.	- - - - -	Spleen and liver lesions	Do.
			83499	- - - - -	do.	+ P P S -	No visible lesions	Positive.
			83410	- - - - -	do.	- - - - -	do.	Negative.
439	40.6	+ P P P - -	83480	- - - - -	do.	S - - - -	do.	Do.
			83481	Mar. 26	- - - - -	(c)	No Bact. abortus lesions	Positive.
			83482	- - - - -	Mar. 20	+ + P P -	No visible lesions	Not cultured
			83483	- - - - -	do.	P P S S -	do.	Positive.
409	15	P P P P - -	83493	- - - - -	do.	- - - - -	do.	Do.
			83494	- - - - -	do.	- - - - -	do.	Negative.
			83495	Mar. 17	- - - - -	(c)	No autopsy	Do.
			83496	Mar. 26	- - - - -	(c)	do.	Not cultured
442	-----	+ + + P P P	83425	Mar. 9	- - - - -	(c)	No Bact. abortus lesions	Do.
			83426	- - - - -	Mar. 29	- - - - -	No visible lesions	Do.
			83427	- - - - -	do.	- - - - -	do.	Negative.
			83470	- - - - -	do.	- - - - -	No visible lesions	Do.
468	42.6	P P S S - -	83471	Mar. 4	- - - - -	(c)	No Bact. abortus lesions	Do.
			83472	Mar. 19	- - - - -	(c)	do.	Do.
			83473	- - - - -	Mar. 29	- - - - -	No visible lesions	Not cultured.
			- - - - -	- - - - -	- - - - -	- - - - -	Negative.	

CONTROLS

17	18	P S S — —	83485 83486 83487 83488	Mar. 10	Mar. 29	— — P P P — —	— — (c) —	No visible lesions. No autopsy. No visible lesions.	Negative. Not cultured. Positive. Negative.
71	7.3	+ + + P P P	83474 83475 83476 83477	do	do	+ + + S — + + + P P — + + + P P — + + + P S —	— — — (c)	Spleen and liver lesions. No visible lesions. Spleen enlarged. No visible lesions.	Negative. Do. Do. Do.
84	32.8	+ S S — —	83478 83479 83480	Mar. 29	Mar. 29	— — —	— — —	No Bact. abortus lesions. No visible lesions.	Negative. Do. Do.
223	Dry.	P + + + + +	83481 83711 83712 83713	do	do	P P P P — S — — — — — — — — —	— — — —	do do do	Positive. Do. Do.
254	Dry.	P P + + + +	83714 83489 83490 83491	do	do	+ P S S S — P P S S S — + P P P P S — + + + P P P —	— — — (c)	Spleen enlarged. Spleen and liver lesions. Spleen enlarged. Enteritis.	Negative. Positive. Do. Do.
441	27.9	+ P — — —	83492 83707 83708 83709	Mar. 27	Mar. 29	— — — —	— — — (c)	No visible lesions.	Not cultured. Negative. Do.
465	7.8	+ + + S — —	83710 83715 83716 83717	Mar. 16	Mar. 29	P S S — — + + + S — — + + + P P S — + + + P S —	— — — —	No Bact. abortus lesions. Spleen and liver lesions.	Do. Do. Positive. Do.
486	26	P P P P — —	83718 83719 83720 83721 83722	do	do	+ + + S — — + + + P P P — + + + S S — — + + + P P P — P P S — —	— — — — —	No visible lesions. Spleen enlarged. Spleen and liver lesions. do No visible lesions.	Do. Do. Do. Do. Do.

^a For the 24 hours (Feb. 19, 1924) just prior to taking samples.
^b All inoculated Feb. 20, 1924, 5 c. c. milk per pig.
^c No test.

TABLE X.—Number of guinea pigs which yielded positive or negative cultural results for *Bacterium abortus* following inoculations with milk from experimental cows in Tables II, IV, V, VI, VII, VIII, and IX

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.*	First group (inoculated Mar. 29, 1922)		Second group (inoculated Aug. 24, 1922)		Third group (inoculated Oct. 10, 1922)		Fourth group (inoculated Dec. 6, 1922)		Fifth group (inoculated May 9, 1923)		Sixth group (inoculated Sept. 27, 1923)		Seventh group (inoculated Feb. 20, 1924)	
	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive
51.....	2			3		3								
247.....	2		2	1	2		3		4		2		1	3
432.....	1	1	2	1		2		2		4		3		3
435.....	2	2	2	1		2	2			4	1	2		3
439.....	1	1	1	2	1	2		3	1	3		3	3	
409.....	1	2	1	2	2	1	1	2	1	3		3		2
442.....	2		2	1		2		3	2	1		2		2
468.....	2		2	1	1	2	3		2	1	2	1		2

CONTROLS

17.....	2			3	1	2		3		4		3	1	2
71.....	1	1	3		3			3	3		3		4	
84.....	1	1		3		3		3		4		3		4
223.....	2	1	1	2	3		2	1	2	2	1	2	3	1
254.....	1	1	3		3		3		2	2	2	1	4	
441.....	2			3		1		3		4		3		3
465.....	3		1	1	2	1	3		1	3	1	2		
466.....	1	1	3		3		3		3		3		4	

* Cow No. 51 died after third test.

TABLE XI.—Agglutination reactions of blood obtained on different dates during the experiments

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Tested Mar. 28, 1922	Tested Aug. 23, 1922	Tested Oct. 9, 1922	Tested Dec. 5, 1922	Tested May 8, 1923
51.....	++++ PSS	+++++S-	++++++P	-----	++++++
247.....	++++++-	++++++	++++++	++++++	++++++PP
432.....	++++++	++++++	PP+ ++	++++++	++++++PP
435.....	+++++PS	SSSSPP	+PPPPPP	+++++SS	+PSSS-
439.....	++++++	PP+ ++	PP+ ++	+++++PP	PPPP++
409.....	++++++	PPPP++	++++++	++++++P	++++++S-
442.....	++++++	PPPP++	+PPPP+P	++++++	P++++++
468.....	SSSSS-	PPPP++S	++++++PP	+++++S-	++++++PP

CONTROLS

17.....	++++PPP	PPSSSS	++++PPP	++++++-	++++SSS-
71.....	++++PPP	PP++++	++++++	++++++P	++++++
84.....	+++++S	+PPPPP	+++++PS	+++++PP	+++++PS-
223.....	++++++	P+++++	++++++-	++++++P	++++++
254.....	++++++	++++++PP	++++++	++++++P	++++++-
441.....	++++++	++++++PP	++++++P	++++++PP	++++++SS
465.....	PPPPPP	P++++P-	PPPPPS	++++++P-	++++++
466.....	PPPPPP-	+PPPPPP	PPPPPS	++++++P-	+PPPPP

TABLE XII.—*Agglutination reactions of milk obtained on different dates during the experiment*
COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Tested Mar. 28, 1922		Tested Aug. 23, 1922		Tested Oct. 9, 1922		Tested Dec. 5, 1922		Tested May 8, 1923		Tested Sept. 26, 1923		Tested Feb. 19, 1924	
	Milk yield, pounds •	Results	Milk yield, pounds •	Results	Milk yield, pounds •	Results	Milk yield, pounds •	Results	Milk yield, pounds •	Results	Milk yield, pounds •	Results	Milk yield, pounds •	Results
51	47	P P S —	18.2	P S —	21.6	P P P S —	21.8	+	18	+	19.8	+	16.1	+
247	49.3	+	—	+	18.5	+	7	+	21	+	8.2	+	Dry.	+
432	24.4	+	+	+	15.4	+	21.1	+	15.9	+	20	+	10.6	+
435	34.6	+	+	+	26	+	16.8	+	11.2	+	2.2	+	40.6	+
439	17.7	+	+	+	20.1	+	14.5	+	37.3	+	24.6	+	15	+
409	34.4	+	+	+	20.7	+	8.9	+	33	+	28.1	+	—	+
442	34	+	+	+	15.1	+	14.3	+	9.9	+	3.6	+	—	+
468	23.1	P S S —	17.2	+	11.8	+	—	—	—	—	—	—	42.6	+
CONTROLS														
17	9.8	P P P S —	18.3	P P P S —	14.6	+	15	+	9.6	+	Dry.	+	18	P S S —
71	42.7	+	+	+	13.2	+	10.4	+	Dry.	+	24.8	+	7.3	+
84	Dry.	+	+	+	18.3	+	17.8	+	10.6	+	1	+	32.8	+
223	—	+	+	+	43.4	+	40.1	+	17.9	+	10	+	Dry.	+
254	44.5	+	+	+	Dry.	+	Dry.	+	51.5	+	34.8	+	27.9	+
441	28.6	+	+	+	11.5	+	16	+	14.3	+	Dry.	+	7.8	+
465	21.8	+	+	+	10.5	+	Dry.	+	24.2	+	14.4	+	—	+
466	24.7	P P P S —	Dry.	+	Dry.	+	34.2	+	19.9	+	Dry.	+	26	+

• The milk yield is for the 24-hour period just prior to taking samples.

TABLE XIII.—*Breeding data on experimental cows during period of experiment*
COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Data
247	Nonpregnant following 12 services.
409	Calved May 1, 1923 (282 days); pregnant; due to calve May 19, 1924.
432	Calved Feb. 28, 1923 (278 days); pregnant; due to calve Apr. 29, 1924.
435	Calved July 6, 1923 (272 days); pregnant; due to calve July 6, 1924.
439	Aborted Sept. 20, 1923 (178 days); calved Dec. 13, 1924 (278 days).
442	Calved Apr. 30, 1923 (272 days); pregnant; due to calve June 14, 1924.
468	Served 10 times; calved Jan. 5, 1924 (276 days).
CONTROLS	
17	Calved June 15, 1922 (calf vigorous); calved Jan. 8, 1924 (265 days).
71	Calved July 17, 1923 (278 days); served Feb. 27, 1924 (probably pregnant).
84	Aborted July 29, 1922 (243 days); aborted Dec. 31, 1923 (252 days).
223	Calved June 19, 1922 (calf vigorous); calved Mar. 9, 1924 (286 days).
254	Calved Jan. 28, 1923 (281 days); pregnant; due to calve May 7, 1924.
441	Calved Oct. 29, 1923 (280 days); served Jan. 23, 1924 (probably pregnant).
465	Calved Jan. 20, 1923 (277 days); served 10 times; last service Jan. 22, 1924.
466	Calved Oct. 21, 1922 (282 days); calved Sept. 28, 1923 (278 days).

The results from the guinea-pig inoculations failed to indicate that the infective properties of the milk of different cows was influenced greatly by the quantity produced. It was also noted that unless the cows were no longer being milked daily, the quantity of milk produced seemed to have no marked influence on the agglutinin titer of the whey. It was observed, however, that the milk of certain cows, whether they were practically dry or producing from 35 to 40 pounds daily, infected guinea pigs with marked regularity when injected in 5 c. c. quantities. Cows 71 and 466 illustrate this point. The milk of other subjects produced less uniform results in this respect. Whether such variations were due to differences in virulence of strains, numbers of organisms contained in the milk samples, or to the resistance of different pigs was not determined.

SUMMARY

Repeated injections of *Bact. abortus* bacterin, in which the organisms had been killed by heat, did not prove to be of practical value in overcoming *Bact. abortus* udder infection in cows.

Eight of a group of fifteen cows with udders infected by *Bact. abortus* produced milk capable of infecting guinea pigs for approximately two years.

Variations in the quantity of milk produced by a *Bact. abortus* udder-infected cow, provided the animal is milked daily, did not markedly affect the agglutination reaction of the milk whey.

The blood serum of cows which have acquired infectious abortion may react strongly to the agglutination test for at least a year after the disappearance of the infection from the udder, as indicated by guinea-pig inoculation tests.

SHAPE AND WEIGHT OF EGGS IN RELATION TO THEIR HATCHING QUALITY¹

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INTRODUCTION

During recent years the annual replenishment of the chicken flocks of the country by means of artificial incubation has been accompanied by excessive embryonic mortality. The problem of the hatchability of hen eggs is therefore an important one, inasmuch as there are a number of factors that may cause the death of a relatively large number of embryos in proportion to the total number of fertile eggs artificially incubated. Concerning the various factors possibly contributing toward this heavy embryonic mortality, the reader is referred to a résumé contributed by Dunn.² There is need for much more investigational work concerning the effects of the various processes of incubation on embryonic development and variations in respect to the inherent hatching quality of eggs.

Concerning the inherent qualities that possibly affect hatchability Dunn has investigated the relationship between the weight and the hatching quality of White Leghorn eggs. Among other things, he found that for both pullets and hens, the largest eggs did not hatch as well as eggs near the average weight for the flock, and that there was no correlation between the mean weight of the eggs laid by an individual pullet or hen and the proportion of her fertile eggs which hatched. If the first result obtained by Dunn applies to other breeds and varieties of chickens as well as to other strains of White Leghorns, then it becomes important to determine to what extent large eggs should be eliminated from the eggs saved for incubation. On the other hand, the second result noted by Dunn implies that the elimination of the eggs laid by individual birds whose mean egg weight is above the mean for the flock will not affect the proportion of fertile eggs hatching. This apparent paradoxical situation is explained by the fact that Dunn also found that among the eggs of an individual fowl those which were above the mean weight for the individual did not hatch as well as those which were below the mean weight, regardless of the absolute mean egg weight.

In order to ascertain the relationship between weight and hatching quality of eggs in Barred Plymouth Rocks and Rhode Island Reds, the writers have gone over the incubation records of a large number of birds, including pullets and hens, and have prepared the data as presented in the following tables. The relationship between shape

¹ Received for publication January 19, 1920; issued December, 1925.

² DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGGS. Conn. Storrs Agr. Exp. Sta. Bul. 109: 92-114. 1922.

and hatching quality of eggs laid by a flock of Barred Plymouth Rock pullets has also been considered, and the data pertaining thereto are presented here.

MATERIAL AND METHODS

The group of birds on which the study of the relationship between egg shape and hatching quality is based consisted of two flocks each of 12 Barred Plymouth Rock pullets mated to Brown Leghorn cockerels. The eggs laid between the first of February and the last of April were dated and carefully measured when laid. The length and maximum breadth of each egg were measured in millimeters, the measurements being recorded to hundredths of a millimeter. The length-breadth index was used as a measure of shape and was obtained by dividing 100 times the breadth by the length. A long and narrow egg has a relatively low index, while a short and broad egg has a high index.

Five groups of birds contributed the material for the study of the relationship between egg weight and hatching quality. The first group consisted of 53 Rhode Island Red hens mated to Rhode Island Red cockerels. The second group consisted of 30 Rhode Island Red pullets mated to Rhode Island Red cockerels. The third group consisted of 50 Barred Plymouth Rock hens mated to Rhode Island Red cockerels. The fourth group consisted of 113 Barred Plymouth Rock pullets mated to Rhode Island Red cockerels. In the case of each of these groups, matings consisted of from 12 to 15 females mated to one male. The fifth group consisted of the two matings of Barred Plymouth Rock pullets with the Brown Leghorn males mentioned. In the case of the first two groups the eggs were laid between March 5 and March 23; the eggs of the second two groups were laid between March 15 and March 23; the eggs of the last group were laid between the first of February and the last of April. The eggs were dated and weighed daily as laid, the weights being recorded to hundredths of a gram.

The eggs of the four largest groups of birds were incubated in a triple-deck mammoth incubator, 5,400-egg capacity, the conditions of the incubation for all the eggs being kept as nearly uniform as possible. The eggs of the fifth group of birds were incubated in an electric incubator of 100-egg capacity, the conditions of incubation also being kept as nearly uniform as possible. All eggs were tested on the seventh day, records being made of the infertile ones and those in which the embryo had died. A second test was made on the fifteenth day, records being made of the embryos which died between the seventh and fifteenth days. On the nineteenth day of incubation the eggs were removed from the incubators, and each egg was placed in a separate hatching sack and replaced in the incubator. On the twenty-second day all hatching sacks were removed from the incubator, and the eggs which hatched were recorded as well as those in which the embryo had died between the fifteenth and the twenty-first days.

In the tables which follow, the data for embryo mortality are divided into two parts, embryos which "died early" and embryos which "died late." The embryos recorded as having died early include all

embryos which died up to and including the fifteenth day of incubation, while those recorded as having died late are the embryos which died after the fifteenth day of incubation.

SHAPE OF EGGS IN RELATION TO HATCHING QUALITY

The evidence on the relationship of egg weight and hatching quality is presented in Tables I, II, and III. In Table I the incubation results of the 1,253 eggs laid by the 24 Barred Plymouth Rock pullets are given, showing for each shape-index class the number of eggs set, the number of infertile eggs, the number which died early, the number which died late, and the number which hatched.

TABLE I.—*Incubation results of eggs for different classes of shape index*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Shape index	Number set	Infertile	Died early	Died late	Hatched
61.00 to 62.99.....	2	2
63.00 to 64.99.....	3	1	1	1
65.00 to 66.99.....	13	2	6	5
67.00 to 68.99.....	33	1	3	10	19
69.00 to 70.99.....	89	4	6	21	58
71.00 to 72.99.....	172	11	14	43	104
73.00 to 74.99.....	279	28	31	61	159
75.00 to 76.99.....	295	35	32	54	174
77.00 to 78.99.....	235	26	23	42	144
79.00 to 80.99.....	85	7	5	19	54
81.00 to 82.99.....	35	1	3	15	16
83.00 to 84.99.....	12	1	2	2	7
Total.....	1,253	115	121	274	743
Mean shape index.....	75.07±0.07	75.54±0.18	75.14±0.20	74.89±0.15	75.05±0.08
Standard deviation.....	3.42±.05	2.92±.13	3.26±.14	3.69±.11	3.40±.06
Coefficient of variability.....	4.56±.06	3.87±.17	4.34±.19	4.93±.14	4.53±.08

Of the 1,253 eggs set, 1,138 (90.82 per cent) were fertile, and 743 (59.29 per cent) hatched. Of the fertile eggs, 121 (10.63 per cent) died early, 274 (24.08 per cent) died late; and 743 (65.29 per cent) hatched.

The mean shape index for the different groups of eggs was as follows: Number set, 75.07±0.07; infertile, 75.54±0.18; died early, 75.14±0.20; died late, 74.89±0.15; hatched, 75.05±0.08. The differences in mean shape index are very slight and are not significant; as, for instance, the difference with its probable error between the mean shape index of the highest value, 75.54±0.18, and the mean shape index of lowest value, 74.89±0.15, is 0.65±0.23. The relative and absolute amounts of variation in shape index, as shown by the standard deviations and the coefficients of variability, are somewhat similar. Judging from the results as shown in Table I it would seem that the shape of an egg does not affect its hatchability.

The percentage of fertile eggs that hatched for each shape-index class in Table I is not shown, however, since the number of eggs set in some of the shape-index classes was very small and comparisons of hatchability between any two classes would not provide a satisfactory basis of determining the possible influence of shape of egg on hatchability. For instance, in the shape-index class 61.00 to 62.99

2 eggs were set, both of which hatched, giving a hatching percentage of 100, while in the shape-index class 63.00 to 64.99 3 eggs were set, 2 of which were fertile and of which only 1 hatched, giving a hatching percentage of 50 for the fertile eggs. That is, a difference of 1 chick in absolute numbers hatched made a difference of 50 per cent of the fertile eggs that hatched.

In Table II all eggs set have been grouped in classes differing by greater increments of shape index than is shown in Table I, the shape-index classes being divided into three groups, low (61.00 to 68.99), medium (69.00 to 76.99), and high (77.00 to 84.99).

TABLE II.—Incubation results of eggs for groups of low, medium, and high shape index classes

[Eggs laid by 24 Barred Plymouth Rock pullets]

Shape index	Number set	Infertile	Died early	Died late	Hatched	Per cent of fertile eggs hatched
61.00 to 68.99.....	51	2	5	17	27	55.10±7.68
69.00 to 76.99.....	835	78	83	179	495	65.39±.63
77.00 to 84.99.....	367	35	33	78	221	66.60±2.75

Of particular interest in Table II is the per cent of fertile eggs hatched in each of the three groups. The low shape-index group shows a hatching percentage of 55.10 ± 7.68 , the medium shape-index group 65.39 ± 0.63 , the high shape-index group 66.60 ± 2.75 . The difference in hatchability between the low and medium groups is 10.29 ± 7.70 and is therefore not significant. The difference in hatchability between the medium and high groups is 1.21 ± 2.82 , and also is not significant. The difference in hatchability between the low and high groups is 11.50 ± 8.16 , and is not significant. It may be concluded, therefore, that so far as the eggs of the flock as a whole are concerned there is no relationship between the shape of an egg and its hatching quality.

To determine the relation between mean shape index and hatching quality within the individual, the data were regrouped in Table III and show for each pullet the number and mean shape index of the eggs whose embryos died in shell and of the eggs which hatched. The eggs whose embryos died early and those whose embryos died late have been grouped together, because in several cases the numbers of each were too small to be considered separately. Moreover, the factor of importance is to determine the relationship in respect to mean shape index between the eggs which failed to hatch and those which hatched, the infertile eggs not being taken into account.

TABLE III.—Mean shape index of eggs whose embryos died in shell and that hatched per bird

[Eggs laid by 24 Barred Plymouth Rock pullets]

Pullet No.	Died in shell		Hatched		Difference
	Num-ber	Mean shape index	Num-ber	Mean shape index	
1.....	20	76.02±0.53	32	76.55±0.16	0.53±0.55
2.....	18	75.38±.38	32	75.23±.15	.15±.41
3.....	17	77.71±.44	42	77.51±.13	.20±.46
4.....	16	76.87±.27	28	76.80±.17	.07±.32
5.....	13	78.55±.67	38	78.38±.14	.17±.68
6.....	13	80.43±.54	33	81.51±.21	1.08±.58
7.....	12	74.01±.77	34	74.30±.20	.29±.80
8.....	14	75.93±.37	27	76.98±.27	1.05±.46
9.....	15	72.48±.62	26	71.97±.14	.51±.64
10.....	18	77.16±.43	31	75.81±.28	1.35±.51
11.....	16	73.21±.31	28	73.01±.16	.20±.35
12.....	13	73.26±.41	35	72.84±.16	.42±.44
13.....	19	74.03±.22	22	74.53±.24	.50±.33
14.....	19	76.99±.20	37	76.26±.13	.73±.24
15.....	22	69.46±.56	28	69.76±.17	.30±.59
16.....	13	70.43±.41	22	71.22±.21	.79±.49
17.....	18	77.24±.28	30	76.62±.19	.62±.34
18.....	18	73.86±.32	29	74.31±.19	.45±.37
19.....	11	75.38±.61	19	73.83±.24	1.55±.66
20.....	18	76.31±.21	37	77.00±.15	.69±.26
21.....	16	77.91±.28	28	77.31±.23	.60±.36
22.....	14	74.03±.31	34	73.18±.14	.85±.34
23.....	18	74.37±.24	37	75.02±.16	.65±.29
24.....	28	78.87±.16	30	78.26±.17	.61±.23

The number of eggs per pullet whose embryos died in shell and those which hatched varied from 30 to 59, with an average of 47.42. The number of eggs whose embryos died in shell varied from 11 to 28 with an average of 16.44 per pullet. The number of eggs which hatched varied from 19 to 42, with an average of 30.98 per pullet.

It is apparent from the data in the last column of Table III that there is no significant difference in any individual pullet between the mean shape index of the eggs whose embryos died in shell and the mean shape index of the eggs which hatched.

The per cent of fertile eggs which hatched has been determined for each of the 24 pullets and the percentages have been correlated with the mean shape index of the fertile eggs of each pullet. The value of this correlation has been found to be 0.27 ± 0.13 . Therefore, the mean shape index of a pullet's eggs does not affect their hatchability.

WEIGHT OF EGGS IN RELATION TO HATCHABILITY

In this study, in addition to the 1,253 eggs dealt with previously, the records of 1,392 other eggs are available, including 534 laid by Rhode Island Red hens, 342 laid by Rhode Island Red pullets, 148 laid by Barred Plymouth Rock hens, and 368 laid by Barred Plymouth Rock pullets.

The data pertaining to the second group of eggs are shown in Table IV. For the sake of comparison, the mean weights of the eggs set, those which were infertile, those which died early, those which died late, and those which hatched are shown in comprehensive form. The per cent of fertile eggs which hatched is also shown for each group and for each breed.

TABLE IV.—Mean weights, classified according to incubation results, of eggs laid by four groups of birds

[Eggs laid by 53 Rhode Island Red hens and 30 Rhode Island Red pullets]

	Rhode Island Red hens		Rhode Island Red pullets		All Rhode Island Reds	
	Num-ber	Mean weight	Num-ber	Mean weight	Num-ber	Mean weight
		<i>Grams</i>		<i>Grams</i>		<i>Grams</i>
Set.....	534	59.82±0.13	342	56.32±0.15	876	58.46±0.11
Infertile.....	76	60.15±.36	12	55.87±.71	88	59.56±.46
Died early.....	37	59.83±.43	13	57.65±.50	50	58.78±.37
Died late.....	100	60.51±.29	65	55.81±.29	165	58.94±.21
Hatched.....	321*	59.64±.17	252	56.36±.17	573	58.20±.14
Per cent of fertile eggs hatched.....	70.08	-----	76.36	-----	72.71	-----

[Eggs laid by 50 Barred Plymouth Rock hens and 113 Barred Plymouth Rock pullets]

	Barred Plymouth Rock hens		Barred Plymouth Rock pullets		All Plymouth Rocks	
	Num-ber	Mean weight	Num-ber	Mean weight	Num-ber	Mean weight
		<i>Grams</i>		<i>Grams</i>		<i>Grams</i>
Set.....	148	60.25±0.23	368	57.57±0.15	516	58.33±0.13
Infertile.....	17	59.90±.79	45	57.64±.39	62	58.26±.37
Died early.....	11	61.50±.66	27	56.76±.42	38	58.13±.41
Died late.....	8	60.64±1.05	67	57.34±.30	75	57.69±.32
Hatched.....	112	60.10±.26	229	57.71±.18	341	58.50±.15
Per cent of fertile eggs hatched.....	85.49	-----	70.90	-----	75.11	-----

The percentage of fertile eggs that hatched were as follows: Rhode Island Red hens, 70.08; Rhode Island Red pullets, 76.36; all Rhode Island Reds, 72.71; Barred Plymouth Rock hens, 85.49; Barred Plymouth Rock pullets, 70.90; all Barred Plymouth Rocks, 75.11.

It is apparent, from an examination of the mean weights of eggs in each group of birds and in each breed, that there is no significant difference between the mean weights of the eggs whose embryos died either early or late and the mean weights of the eggs that hatched. Apparently, then, in these groups of birds, the relative size of egg has had no effect on hatching quality. Dunn³ found, however, "an evident tendency for eggs which die during incubation to be slightly larger than those which hatch." Such a situation is not evident in the data shown in Table IV, and in order to test the matter conclusively the more homogeneous lot of 1,253 eggs laid by 24 Barred Plymouth Rock pullets is analyzed.

In Table V are shown the frequency distributions and the mean weights of the eggs set, those which were infertile, those in which the embryos died early, those in which the embryos died late, and those which hatched. In each case the frequency distributions follow fairly closely a normal curve. There are no significant differences between the mean weights of any two groups of eggs. The mean weight in grams is greater in the eggs whose embryos died early, 55.51 ± 0.18 ,

³ DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGG
Conn. Storrs Agr. Exp. Sta. Bul. 109, p. 100. 1922.

while among the fertile eggs it is lowest in the eggs which hatched, 55.25 ± 0.10 . The difference in weight between these two groups is 0.26 ± 0.21 , which is not significant. The greatest variation is among the infertile eggs, while among the fertile eggs the relative and absolute amounts of variation are much the same in the three groups. Here, again, as in the case of the other four groups of birds, there is no evidence of any significant differences among the weights of eggs whose embryos died early, those whose embryos died late, and those which hatched.

TABLE V.—*Incubation results of eggs for different weight classes*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Weight	Number set	Infertile	Died early	Died late	Hatched
<i>Grams</i>					
46.00 to 46.99.....	2	-----	-----	1	1
47.00 to 47.99.....	4	1	-----	1	2
48.00 to 48.99.....	7	2	1	1	3
49.00 to 49.99.....	11	2	1	3	5
50.00 to 50.99.....	13	3	3	2	5
51.00 to 51.99.....	28	4	2	5	17
52.00 to 52.99.....	77	7	6	14	50
53.00 to 53.99.....	164	12	14	32	106
54.00 to 54.99.....	218	19	22	48	129
55.00 to 55.99.....	227	21	20	52	134
56.00 to 56.99.....	196	17	16	42	121
57.00 to 57.99.....	168	11	20	43	94
58.00 to 58.99.....	81	8	7	16	50
59.00 to 59.99.....	29	3	4	8	14
60.00 to 60.99.....	13	2	4	3	4
61.00 to 61.99.....	6	1	1	1	3
62.00 to 62.99.....	3	1	-----	-----	2
63.00 to 63.99.....	2	-----	-----	1	1
64.00 to 64.99.....	1	-----	-----	-----	1
65.00 to 65.99.....	2	1	-----	-----	1
66.00 to 66.99.....	1	-----	-----	1	-----
Total.....	1, 253	115	121	274	743
Mean weight.....	55. 28±0. 04	55. 13±0. 18	55. 51±0. 15	55. 31±0. 10	55. 25±0. 05
Standard deviation.....	2. 22± . 03	2. 82± . 13	2. 37± . 10	2. 35± . 07	2. 22± . 04
Coefficient of variability.....	4. 20± . 06	5. 12± . 23	4. 27± . 19	4. 25± . 12	4. 02± . 07

The same data as shown in Table V are grouped in Table VI in three classes of weight increments, low (46.00 to 52.99 grams), medium (53.00 to 59.99 grams), and high (60.00 to 66.99 grams).

TABLE VI.—*Incubation results of eggs for groups of low, medium, and high weight classes*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Weight	Number set	Infertile	Died early	Died late	Hatched	Per cent of fertile eggs hatched
<i>Grams</i>						
46.00 to 52.99.....	142	19	13	27	83	67. 48±2. 04
53.00 to 59.99.....	1, 083	91	103	241	648	65. 32±1. 35
60.00 to 66.99.....	28	5	5	6	12	52. 17±9. 46

The low-weight group contains a slightly larger percentage of fertile eggs that hatched than the medium-weight group, while the high-weight group contains a percentage of fertile eggs that hatched considerably lower than either of the others. This situation is much the same as that reported by Dunn ⁴ for White Leghorn pullets, except that his medium-weight group (52.00 to 59.99 grams) contained the largest percentage of fertile eggs that hatched, although the difference in hatchability between his low (eggs weighing up to 51.99 grams) and medium-weight groups was not significant. The difference in percentage of hatchability between the low- and medium-weight groups in this study on Barred Plymouth Rocks is 2.16 ± 2.45 ; between the medium- and high-weight groups it is 13.15 ± 9.55 ; between the low- and high-weight groups it is 15.31 ± 9.67 . None of these differences in percentage of fertile eggs hatched are significant, whereas Dunn obtained significant differences between his medium- and high-weight groups and between his low- and high-weight groups. Briefly, then, in Dunn's White Leghorn pullets the larger eggs appear to have been somewhat less hatchable than those of small and medium size, while in the case of eggs laid by the Barred Plymouth Rocks used in this study the larger eggs also gave a lower hatching percentage, but no significance is attached to it.

Up to the present, the matter of a possible relationship between egg weight and hatching quality has been considered from the standpoint of the eggs from the flock as a whole. Since the results of several lines of investigation have shown, however, that variation in hatching quality of eggs has been found to be chiefly a variation among individual fowls, it becomes necessary to consider the data shown in Tables V and VI on the basis of the mean weight of each pullet's eggs whose embryos died in shell and those which hatched, in relation to the percentage of fertile eggs which hatched. In other words, is there any correlation between the mean weight of the eggs of individual pullets and the percentage of their fertile eggs which hatched? To show something on this point, the mean weight of the eggs whose embryos died in shell and the mean weight of those which hatched for each pullet are given in Table VII.

⁴ DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGGS. Conn. Storrs Agr. Exp. Sta. Bul. 109: 92-114. 1922.

TABLE VII.—Mean weight of eggs whose embryos died in shell and that hatched per bird

[Eggs laid by 24 Barred Plymouth Rock pullets]

Pullet No.	Died in shell		Hatched		Difference
	Num-ber	Mean weight	Num-ber	Mean weight	
		<i>Grams</i>		<i>Grams</i>	
1-----	20	55.28±0.23	32	54.69±0.16	0.59±0.28
2-----	18	54.64±.18	32	54.91±.12	.27±.22
3-----	17	54.26±.21	42	54.50±.16	.24±.26
4-----	16	54.28±.20	28	54.32±.17	.04±.26
5-----	13	55.25±.13	38	55.32±.14	.09±.19
6-----	13	55.37±.16	33	55.25±.12	.12±.20
7-----	12	55.43±.24	34	55.65±.14	.22±.28
8-----	14	54.44±.21	27	54.31±.18	.13±.28
9-----	15	58.43±.28	26	57.74±.13	.69±.31
10-----	18	57.60±.17	31	58.21±.14	.61±.22
11-----	16	54.45±.16	28	54.24±.16	.21±.23
12-----	13	56.56±.18	35	56.90±.11	.34±.21
13-----	19	56.65±.17	22	57.26±.21	.61±.27
14-----	19	56.68±.15	37	56.51±.10	.17±.18
15-----	22	57.17±.21	28	57.36±.11	.19±.24
16-----	13	55.23±.18	22	55.10±.21	.13±.28
17-----	18	55.52±.14	30	55.34±.14	.18±.20
18-----	18	54.90±.15	29	55.27±.16	.37±.22
19-----	11	54.88±.20	19	54.41±.21	.47±.29
20-----	18	54.89±.14	37	54.96±.13	.07±.19
21-----	16	55.33±.16	28	55.18±.12	.15±.20
22-----	14	55.46±.18	34	55.32±.11	.14±.21
23-----	18	54.34±.16	37	54.85±.12	.51±.20
24-----	28	55.10±.13	30	54.66±.11	.44±.17

The average number of fertile eggs per pullet was 47.42, with a range of from 30 to 59 eggs. The average number of eggs whose embryos died in shell was 16.44 per pullet, with a range of from 11 to 28 eggs. The average number of eggs which hatched was 30.98 per pullet, with a range of from 19 to 42 eggs.

The mean weight of fertile eggs per pullet varied from 54.30 grams to 57.95 grams, with a mean weight of 55.29 ± 0.05 grams for all pullets. The mean weight of the eggs whose embryos died in shell varied from 54.26 grams to 58.43 grams per pullet, with a mean weight of 55.38 ± 0.08 grams for all pullets. The mean of the mean weights in grams of the eggs whose embryos died in shell was 55.50 ± 0.61 per pullet. The mean weight of the eggs which hatched varied from 54.24 grams to 58.21 grams per pullet, with a mean weight of 55.25 ± 0.05 grams for all pullets. The mean of the mean weights of the eggs which hatched was 55.51 ± 0.64 grams per pullet. Considering the means of the mean weights in grams per pullet of the eggs whose embryos died in shell and those which hatched, it is seen that there is very little difference, 55.50 ± 0.61 and 55.51 ± 0.64 . The difference in grams, with its probable error between these two means is 0.01 ± 0.88 , which, of course, is not significant. Also, the difference in grams for all pullets between the mean weight of the eggs whose embryos died in shell, 55.38 ± 0.08 , and the mean weight of the eggs which hatched, 55.25 ± 0.05 , is 0.13 ± 0.09 , which is not significant.

The mean weight in grams of the fertile eggs, and the per cent of fertile eggs which hatched per pullet, have been correlated for the group of 24 pullets. The correlation has been found to be 0.25 ± 0.13 . There is a slight negative correlation, but it is not significant.

SUMMARY

The results of this study of the shape and weight of eggs in relation to hatching quality, using eggs laid by Barred Plymouth Rock pullets and hens and Rhode Island Red pullets and hens, seem to justify the following conclusions:

Egg shape, where normal eggs are involved, does not affect hatching quality.

There are no significant differences in the mean shapes of eggs whose embryos die up to the fifteenth day, those whose embryos die between the fifteenth and twenty-second days of incubation, and those which hatch.

There is no significant correlation between the mean shape of the eggs laid by an individual bird and the proportion of her fertile eggs which hatch.

The selection of eggs for incubation purposes according to shape can not be expected to affect the hatching results.

Egg weight, where normal eggs are involved, has no bearing on hatching quality.

There are no significant differences in the mean weights of eggs whose embryos die up to the fifteenth day, those whose embryos die between the fifteenth and twenty-second days of incubation, and those which hatch.

There is no significant correlation between the mean weight of the eggs laid by an individual bird and the proportion of her fertile eggs which hatch.

The selection of eggs for incubation purposes according to size can not be expected to affect the hatching results.

RELATION BETWEEN THE BACTERIAL COUNT OF WHOLE MILK AND THAT OF THE CREAM AND SKIM MILK SEPARATED FROM IT¹

By C. S. LEETE

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INTRODUCTION

In studying the milk ordinances of many municipalities it is found that when a maximum bacterial count for milk is designated, a much higher maximum bacterial count for cream of the same grade is allowed. This increase covers a wide range, varying usually from 300 to 500 per cent. A review of the literature upon this subject shows that but little work has been done, or at least few results have been published, dealing with the effect of separation upon the bacterial count of cream and skim milk. It would seem that the maximum bacterial standards for cream appearing in many milk ordinances are not based on scientific facts.

The Bureau of Dairying of the United States Department of Agriculture has been carrying on work with centrifugal and gravity separators in order to determine what effect separation has upon the bacterial count of the cream and skim milk.

CENTRIFUGAL SEPARATION

A steam-turbine separator with a capacity of 1,350 pounds per hour was used. It was run at its rated capacity. After use, the separator parts were cleaned with hot water containing a strong washing powder, then thoroughly rinsed with hot water, and steamed for three minutes over an open steam jet. After cooling, the parts were assembled, again steamed, and then placed in a clean inclosed cabinet until the next run.

After the separator had attained its proper speed, mixed-herd milk at a temperature between 80° and 85° F. was separated. Samples of the whole milk, cream, and skim milk were taken after separation had been in progress about three minutes. The milk sample was taken at the outlet of the supply bowl, and the cream and skim milk samples from their respective spouts. The samples for bacteriological analysis were immediately plated. Standard methods for bacteriological analysis of milk, as recommended by the American Public Health Association,² were followed. Plates were incubated for 48 hours at 37.5° C. Fat determinations were made with the Babcock fat test. Samples were taken from 100 separate runs of

¹ Received for publication Jan. 23, 1925; issued December, 1925.

² AMERICAN PUBLIC HEALTH ASSOCIATION AND ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. STANDARD METHODS OF MILK ANALYSIS. BACTERIOLOGICAL AND CHEMICAL. Ed. 4, 40 p., illus. New York City. 1923.

the separator. The average bacterial count of 100 samples of whole milk was 435,240 per cubic centimeter. The cream and skim milk resulting from the separation of the milk gave average bacterial counts of 500,830 and 312,740 per cubic centimeter, respectively. The average per cent butterfat of the whole milk was 3.905, and that of the cream 34.64. The per cent fat in the skim milk was 0.02. The average increase of the bacterial count of all the cream samples over the milk from which it was obtained was 14.84 per cent. Twenty-three of the 100 samples of cream showed a lower count than the whole milk. The per cent decrease for these samples was 14.06. Seven samples gave the same count for the cream as for the whole milk. The increase in 70 samples of cream showing an increase over whole milk was 23.87 per cent. (See Table I.)

TABLE I.—Increase and decrease of bacteria per cubic centimeter in cream as compared with whole milk

	Per cent
Increase in bacteria per cubic centimeter of 100 samples of cream over the whole milk from which the cream was separated.....	14. 84
Increase in bacteria per cubic centimeter of the 70 samples of cream showing increase.....	23. 87
Decrease in bacteria per cubic centimeter of the 23 samples of cream showing decrease.....	14. 06
Seven samples gave same count as whole milk.	

Both high and low count milk was used. The percentage of fat varied from 3.4 per cent to 5.0 per cent. The bulk of the samples gave a bacterial count between 25,000 and 100,000 bacteria per cubic centimeter. (See Tables II and III.)

TABLE II.—Range and average of bacterial counts and butterfat determinations in 100 samples of whole milk and in the cream and skim milk separated from it by centrifuge

	Bacteria per cubic centi- meter		Percentage of butterfat	
	Range	Average	Range	Average
Whole milk.....	9, 000-14, 410, 000	435, 240	3. 4- 5	3. 905
Cream.....	7, 000-18, 600, 000	500, 830	17. 5-54	36. 64
Skim milk.....	9, 000- 7, 500, 000	312, 740		

TABLE III.—Samples grouped according to bacterial counts

	Bacteria per cubic centimeter						
	10, 000 and lower	10, 001 to 25, 000	25, 001 to 50, 000	50, 001 to 100, 000	100, 001 to 500, 000	500, 001 to 1, 000, 000	1, 000, 001 and higher
Number of samples:							
Whole milk.....	4	13	38	20	8	7	9
Cream.....	2	16	33	22	11	7	9
Skim milk.....	4	29	27	13	8	4	9

When the same samples are grouped according to their bacterial count, it appears that the greatest per cent of increase in the cream

over the whole milk occurs with milk of low bacterial count. The greatest per cent of decrease also occurs with low-count milk. Part of this variation is possibly due to limits of laboratory error, which would be more pronounced in a low-count than in a high-count milk when figuring percentage increases and decreases.

Table IV gives the grouping of the samples according to bacterial count, together with the percentage relationship of the counts of the cream and whole milk by bacterial groups.

TABLE IV.—Per cent increase and decrease of bacterial counts of cream and whole milk, classified by grouping of whole milk bacterial count

Bacteria per cubic centimeter	Increase in cream over whole milk		Decrease in cream under whole milk		Number of samples showing no change
	Number of samples	Average per cent	Number of samples	Average per cent	
0 to 50,000.....	37	28.07	12	15.907	6
50,001 to 100,000.....	17	25.2	3	13.13	1
100,001 to 500,000.....	4	14.82	4	14.722	
500,001 to 1,000,000.....	4	10.245	3	9.396	
1,000,001 and over.....	8	10.936	1	6.06	
Total.....	70		23		7

Out of a total of 70 samples showing an increase in the bacterial count of the cream over the whole milk, 64 samples (or 91.4 per cent) gave an increase of 50 per cent or less. In no instance was there a greater increase than 90 per cent. All of the 23 cream samples which showed a decrease in the count under that of the original whole milk gave a decreased count of 50 per cent or less.

From the work which has been presented, it would seem that there is no basis of fact for the assumption that the bacterial count of centrifugally separated cream should be several hundred per cent higher than the whole milk from which it is derived, provided the separator is clean.

GRAVITY SEPARATION

For this work a gravity separation can 18 inches high and 8½ inches in diameter was used. At the bottom was placed a pet cock to draw off the skim milk. A small strip of glass was inserted in the side, extending upward from the bottom of the can sufficiently so that the dividing line between the cream and skim milk could be observed. As a means for holding the milk in the separator at a low temperature, the can was placed in a large tank with sufficient ice and water around it to extend above the depth of the milk in the can.

Before the separator can was used it was thoroughly washed with hot water containing a strong solution of washing powder, then thoroughly rinsed, and finally inverted over a steam jet and allowed to steam for five minutes. The cover was cleaned and steamed in the same manner. After steaming, the cover was placed on the can and the separator was allowed to cool. Fresh mixed-herd milk was then placed in the separator. Samples for bacterial count and fat content were taken. The can was then set into the cooling and

storage tank. The samples for bacterial count were suspended in the ice water, thus assuring that storage conditions would be the same for both the whole-milk sample and the separator can. At the end of 24 hours the separator can was taken out and the skim milk drawn off through the pet cock. Samples for bacterial count and fat test were taken. The cream was then drawn off and samples taken, all being 24 hours old. The average temperature at the beginning of the holding time was 38.44° F. At the end of 24 hours, when the skim milk and cream were drawn off, the average temperature was 45.88° F. The highest individual temperature was 52° F.

Bacterial counts and fat determinations were made the same as with the centrifugal separation samples. Twenty-five lots of milk were separated by this method.

In no case was it found that the cream had a lower count than the whole milk. Two out of twenty-five samples of skim milk showed an increase in the bacterial count over that of the whole milk, while 23 samples gave a lower count. The average counts per cubic centimeter of the 25 samples were: Whole milk, 135,880; cream, 283,680; skim milk, 33,556.

The average per cent increase in bacterial count of the cream over the whole milk was 160.86 per cent. The average fat percentages were: Whole milk, 4.208; cream, 20.52; skim milk, 1.22.

The bacterial counts in the milk varied from 2,000 to 850,000 per cubic centimeter. The majority of the samples gave a bacterial count of 100,000 or lower. The average percentage of butterfat was 4.208, but two samples were 5.0 per cent or higher. (See Tables V and VI.)

TABLE V.—Range and average of bacterial counts and butterfat determinations in 25 samples of whole milk and in the cream and skim milk separated from it by gravity

	Bacteria per cubic centimeter		Percentage of butterfat	
	Range	Average	Range	Average
25 samples whole milk.....	2,000- 850,000	135,880	3.0- 5.8	4.208
25 samples cream.....	5,000-1,700,000	283,680	14.0-24.0	20.52
25 samples skim milk.....	900- 180,000	33,556	0.5- 2.0	1.22

TABLE VI.—Samples grouped according to bacterial count

	Bacteria per cubic centimeter						
	10,000 and under	10,001 to 25,000	25,001 to 50,000	50,001 to 100,000	100,001 to 500,000	500,001 to 1,000,000	1,000,001 and over
Number of samples:							
Whole milk.....	4	7	4	3	4	3	0
Cream.....	1	1	7	4	7	2	3
Skim milk.....	14	4	1	4	2	0	0

In contrast with centrifugally separated cream, gravity separated cream shows a much higher percentage increase in bacterial count over whole milk. The low-count milk gives the greatest increase in bacterial count in the cream. Table 7 shows the groupings of the milk according to bacterial counts, together with percentage increase of cream over whole milk of each group.

TABLE VII.—*Per cent of increase in bacterial count of gravity cream over that of whole milk, classified by groupings of whole-milk bacterial count*

Bacterial count of whole milk	Number of samples	Average per cent of increase	Number of samples showing no change
0 to 50,000.....	15	198.96	0
50,001 to 100,000.....	3	156.34	0
100,001 to 500,000.....	4	89.88	0
500,001 to 1,000,000.....	3	95.53	0
1,000,001 and over.....			
Total.....	25		0

CONCLUSIONS

Centrifugal separation with a clean separator will not result in cream having a greatly higher bacterial count than the whole milk from which the cream is obtained.

Gravity separated cream will give a much higher bacterial count than the whole milk from which it comes.

In view of the fact that gravity cream plays a very minor part in the market-cream trade, it would seem that those milk ordinances allowing cream of a certain grade with a bacterial count hundreds per cent greater than milk of the same grade are based upon custom rather than scientific investigations. High counts in market cream may very probably be due to a poor quality of milk used for separation and to lack of care in sterilizing equipment rather than to any normal causes involved in the process of separation.



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LEAFSPOT OF MAIZE CAUSED BY *OPHIOBOLUS HETEROSTROPHUS*, N. SP., THE ASCIGEROUS STAGE OF A *HELMINTHOSPORIUM* EXHIBITING BIPOLAR GERMINATION¹

By CHARLES DRECHSLER

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INTRODUCTION

For several years the writer has received for identification occasional lots of graminaceous material attacked or suspected of being attacked by members of the genus *Helminthosporium*. Among the more noteworthy of these are certain collections of maize (*Zea mays* L.) leaves affected with a foliar disease, which, with the parasite responsible for it, this writer has briefly described in an abstract (3)² and which is more adequately treated in this paper. While the fungus has evidently not escaped observation by previous writers, it has generally been confused with a congeneric form responsible for leaf blight of maize. Additional interest attaches to the fungus under consideration in that it readily yields an ascigerous stage of a type not hitherto recorded as associated with any species of *Helminthosporium*. The discovery of this association is presumably not without significance in relation to the taxonomic position of a numerous series of apparently allied forms assigned to the genus, including, for example, the parasites causing spotblotch of barley, eyespot of sugar cane, and leafspot of rice, of which the mode of conidial germination by the production of a polar germ tube from one or both end segments represents a common characteristic.

SYMPTOMS

Liberal collections of diseased maize leaves made by A. C. Foster near Sanford, Fla., on September 22, 1923, and by C. Welles near Los Baños, Philippine Islands, in November, 1921, very largely provided the material upon which the present studies were based. The leaves bore numerous lesions, varying considerably in extent, the smaller and evidently incipient ones scarcely discernible and measuring approximately 0.5 mm. in each direction, the larger up to 15.0 mm. in length and from 1.0 to 3.0 mm. in width (fig. 1, A). In the dried condition the interior of the larger spots approximated, on the whole, the cinnamon-buff of Ridgeway's color chart, although this hue was interrupted by rather delicate transverse reddish-brown bands at intervals of 1.0 to 1.5 mm., resulting in a perceptibly zonate appearance. Reddish-brown coloration was equally present also at the margin of the lesion, setting off the latter from the healthy tissue

¹ Received for publication Nov. 29, 1924; issued December, 1925.² Reference is made by number (italic) to "Literature cited," p. 726.

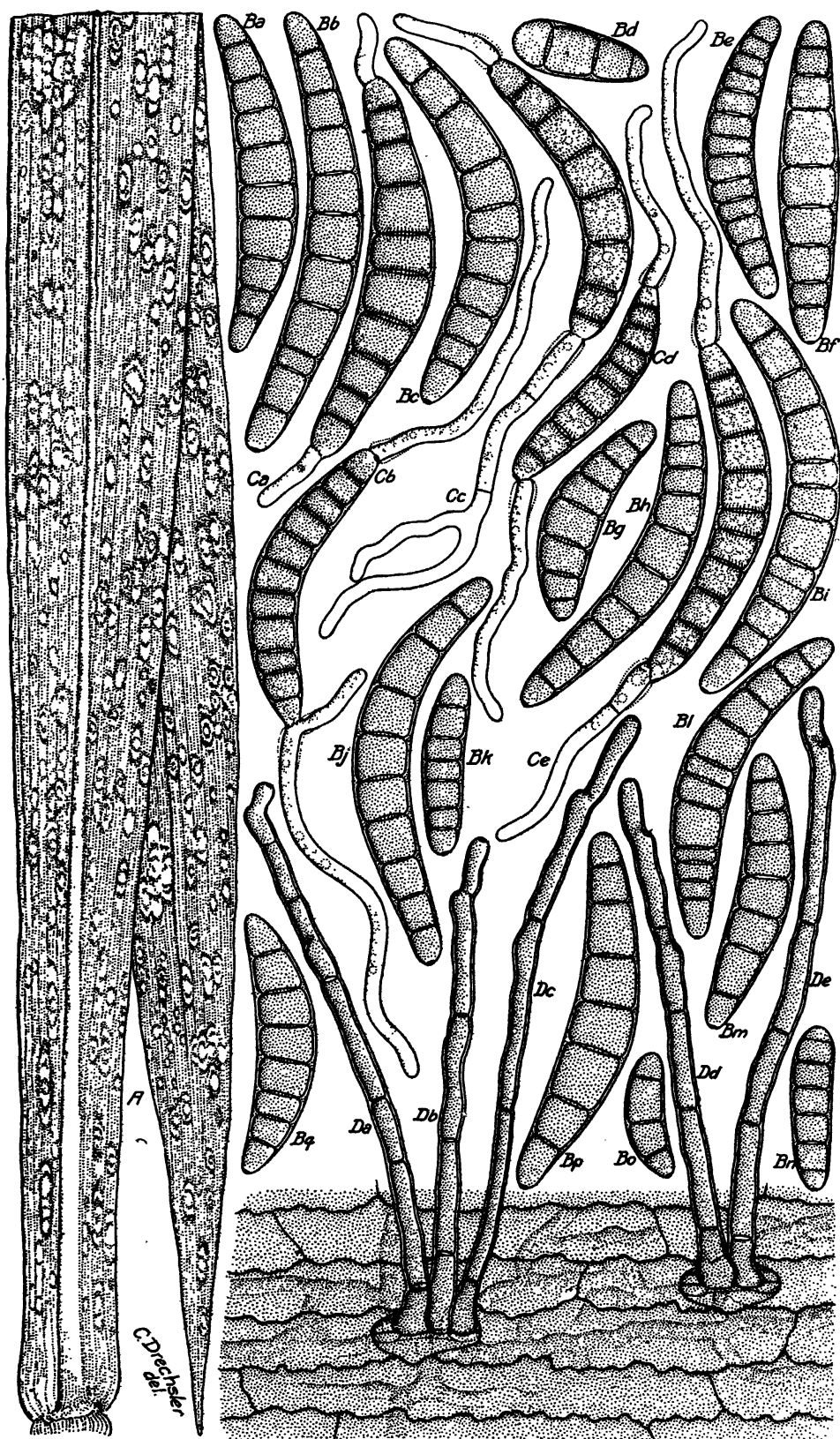


FIG. 1.—A, leaf of maize infected with *Ophiobolus heterostrophus*, showing abundance and size of lesions in specimens collected near Sanford, Fla., Sept. 22, 1923. $\times 7$
 Ba-Bq, conidia of *O. heterostrophus* produced on diseased maize leaves in damp chamber at summer temperature (25 to 30° C.) $\times 474$
 Ca-Ce, conidia germinating by production of 2 polar germ tubes. $\times 474$
 Da-De, conidiophores emerging from stomata as found on material collected in the field. $\times 474$

surrounding it. On some of the less mature foliage a purple tint was frequently evident in lesions that approached the Prussian Red of Ridgway's series. All except the very smallest lesions were elongated in a longitudinal direction, usually with the lateral edges characteristically straight and parallel and coinciding accurately with the course of the veins. Since the latter in the Florida material were spaced at intervals of 1.0 to 1.2 mm., and in the Philippine specimens at intervals of 2.0 to 3.0 mm., the lesions appeared generally as ribbonlike areas of corresponding width. In occasional instances two adjacent intervascular spaces were included in the dead regions and these consequently were double the usual width. The larger regions of discolored foliar tissue, however, had their origin less frequently in such lateral extension of single lesions than in the coalescence of numbers of lesions, side by side and end to end. Owing to the abundance of the spots, which numbered from 200 to 300 on leaves not more than 50 cm. long and 2.5 cm. wide, the morbid parts naturally constituted a considerable portion of the foliar tissue.

Under macroscopic inspection, the appearance of the leaves thus affected does not closely resemble that of specimens affected with blight, a well-known disease widely distributed in maize-producing countries and caused by *Helminthosporium turcicum* Passerini. The blight lesions are relatively few in number, usually not exceeding a dozen to an individual leaf, but their paucity is more than compensated for by their size; they are, when well developed, more than a hundred times larger than even the largest seen in the Florida material. A comparison of Figure 1, A, with Plate 24, A, in a previous publication of the writer (2) suggests the difference in number and size of the two types of lesions. Leaf-blight lesions show no indication of zonation other than a reddish-brown marginal border which often is not well developed or is even entirely suppressed. And while the portions of leaf killed by *H. turcicum* are also elongated longitudinally and often bounded on the sides by the veins of the host, more than one intervascular region are usually involved, the veins apparently providing a much less effective barrier to lateral extension than is evidenced in the specimens under consideration.

CONIDIAL STAGE OF THE CAUSAL PARASITE

Macroscopic examination revealed conidiophores and conidia typical of the genus *Helminthosporium* scattered rather sparsely over the central portions of the larger lesions, the constant positional association strongly suggesting the causal relation subsequently established by inoculation experiments. On the material as obtained from the field, the conidiophores exhibited no unusual peculiarity. Singly, or in groups of two or three, they were found to emerge from the stomata (fig. 1, *Da* to *De*), resembling in the latter detail the homologous structures of *H. turcicum*; although such limitation to stomata is regarded as due to the mechanical firmness of the maize epidermis rather than to an inherent characteristic of the parasites concerned. However, a conspicuous difference distinguishing them from the conidiophores of the leaf-blight fungus was their smaller diameter, in which dimension they were found to measure usually from 4.5 to 7.0 μ instead of 7.5 to 9.0 μ recorded for the other form. In length the conidiophore of the field material measured between 120 and 170 μ , while scars marking the former points of attachment

of spores occurred at fairly wide intervals (10 to 40 μ) at geniculations usually not very pronounced.

Owing to the meager development of the fungus on the material obtained in the field, some of the diseased leaves were incubated in a damp chamber. A luxuriant growth of fructifications at once ensued, with the result that at the end of three days the leaves were covered with a profusion of conidiophores bearing an abundance of fresh conidia (fig. 1, *Ba* to *Bq*). Pure, single-spore cultures of the fungus were obtained by the usual method of transferring individual conidia germinating in poured plates of plain agar to cornmeal agar plates, and then transferring portions of the resultant growth, free of contaminating forms, to cornmeal agar in tubes.

The conidiophores developed in a moist atmosphere on the natural substratum, as in related forms, are not sharply differentiated from the sterile hyphae. Usually they consist of branches or prolongations of subhyaline or fuliginous mycelial filaments, differing from the latter in having a thickened wall dark olivaceous in color. As on the conidiophores found in nature, they develop spores in rather open arrangement, the individual conidia being attached at relatively wide intervals. Branching is not rare although it is more frequent in the sterile portions of the sporiferous elements. The sporophoric filaments, which often attain a length of 1 mm. or more, present, in mass, a brownish-black macroscopic appearance which may become brownish gray or even light gray, depending upon the quantity of subhyaline vegetative mycelium present as an admixture.

The conidia of the fungus as produced on diseased maize leaves in a damp chamber (fig. 1 *Ba* to *Bq*) or on cornmeal agar in pure culture (fig. 2), are readily distinguished from those of *Helminthosporium turcicum* in shape, dimensions, septation, and basal modification. While the spores of the leaf-blight fungus are typically straight or slightly curved, widest at the middle and tapering markedly toward the ends, those of the leafspot parasite are typically strongly curved, the curvature being indeed more pronounced than in any large-spored congeneric form studied by the writer, and tapering moderately toward the ends. In respect to size, the conidia of the new foliar parasite appear markedly inferior to those of the other, measuring only 30 to 115 μ in length and 10 to 17 μ in diameter as compared with 45 to 142 μ and 15 to 25 μ for the ranges of the corresponding dimensions in *H. turcicum*. In spite of its inferior length, however, the conidia of the leafspot fungus are the more abundantly septate, the number of cross-walls not infrequently reaching the observed maximum of 12, while in all material of *H. turcicum* examined by the writer, these have never been seen to exceed 8. An additional difference exists, inasmuch as in the fungus under consideration the hilum is represented by a broad flat scar not generally protruding from the rounded contour of the basal end of the conidium, while the basal segment of the spore of *H. turcicum* exhibits a narrow protruding apiculum. With respect to coloration the distinction is less marked, although the light or moderate olivaceous color characteristic of the mature conidia of the leafspot fungus is noticeably darker than the fuliginous tinge generally present in the conidia of the leaf blight parasite. The same mode of germination by the production of two germ tubes, one from the apical end, the other from the region surrounding the hilum, prevails in both species (fig. 1, *Ca* to *Ce*).

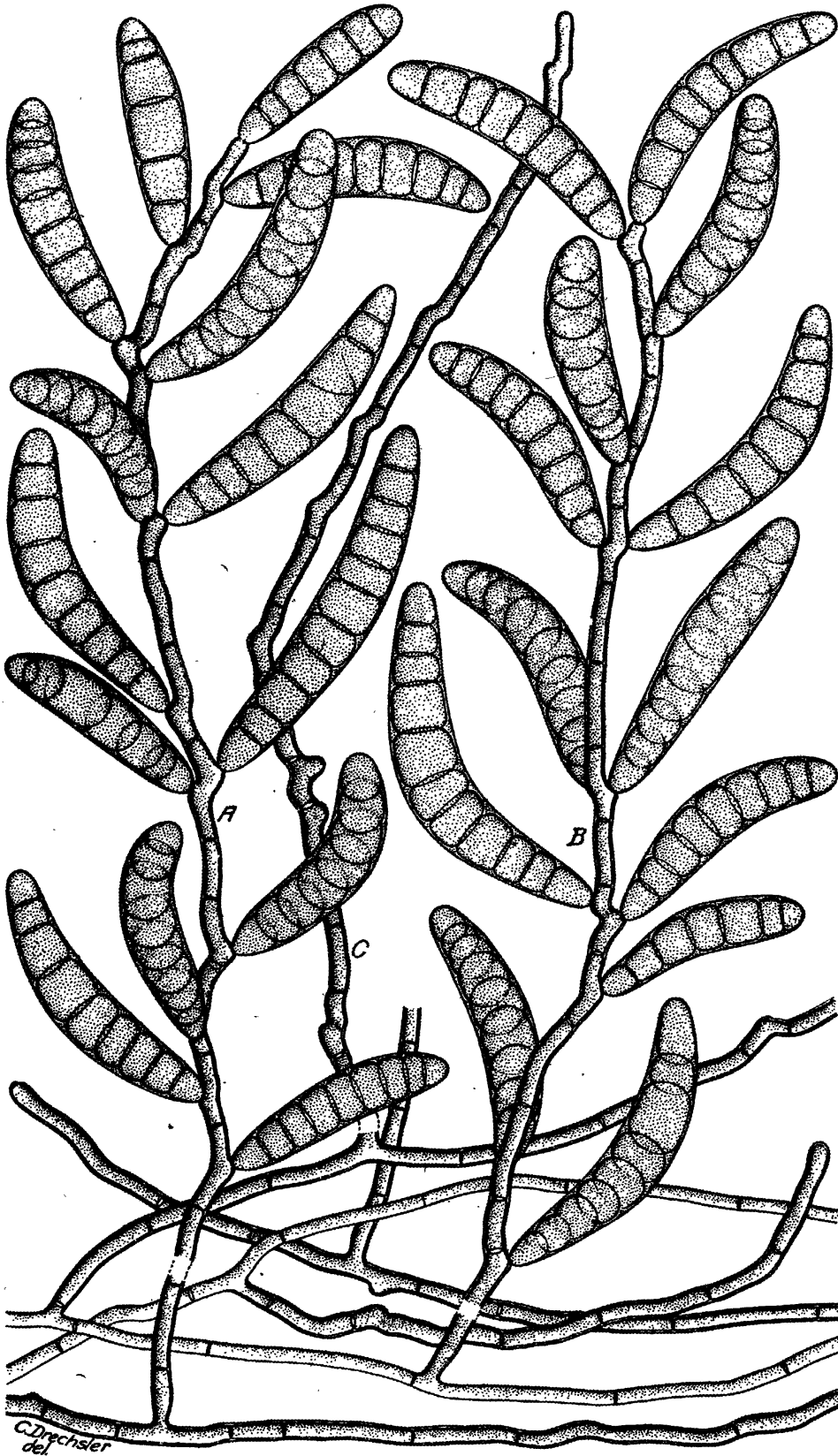


FIG. 2.—Conidiophores and conidia of *Ophiobolus heterostrophus* developed on 30-day-old cornmeal agar plates, arising from somewhat prostrate sporophoric filaments. The dotted lines represent sections of the sterile basal portions of the sporophores omitted for lack of space. X475

DISTRIBUTION ON MAIZE

In this connection, it may not be amiss to consider some passages in the writings of several investigators working on leaf blight in tropical or subtropical regions, which can scarcely be regarded as aptly descriptive of *Helminthosporium turcicum*. Reinking (10), working in the Philippines, described the conidia of *H. inconspicuum* C. and E. (= *H. turcicum* Pass.) as "large, septate, curved brown," a characterization that in regard to coloration and curvature is not generally appropriate. Two figures—one (10, fig. 40 B) representing a conidium from a maize tassel, the other (10, fig. 40, lower c) representing a conidium from a maize leaf—especially deserve comment. The former is shown as a slightly curved structure with nine cross-walls, diminishing only slightly toward the abruptly rounded end, and, from calculations of the size of the figure and the scale of magnification, measuring approximately $112\ \mu$ in length and $17\ \mu$ in diameter; the latter is more strongly curved, provided with 10 septa, tapering somewhat more strongly toward the rounded ends, and measuring approximately $128\ \mu$ in length by $17\ \mu$ in diameter. The spores would appear to be too slender relative to their length, too abundantly septate, and not tapering toward the ends sufficiently to be typical of the leaf-blight fungus. Of the other figures given by the same author, 41b would evidently seem to represent conidia of *H. turcicum*, while 40 upper c as well as 43 appear to pertain to this species, though with less certainty. And the habit figure undoubtedly represents the extensive foliar lesions characteristic of leaf blight.

The descriptive passages in Marquez' recent paper (7) on leaf blight of corn, incorporating the results of studies carried out at Los Baños, are not free of the same sort of ambiguity.

Since the lesions of both leaf blight and leafspot are elongated in a longitudinal direction and are apt to coalesce, the description of the earlier stages may have been drawn from specimens representing either type or probably both types of infection. The leafspot lesion with its parallel lateral boundaries coinciding with two consecutive veins of the host are more accurately described as "stripes" than the effuse areas killed by leaf blight. They also coalesce with much greater frequency, indeed not attaining any considerable size unless abundant coalescence takes place, since the greatest individual lesions rarely exceed 3.0 mm. in width and 15.0 mm. in length. The leaf blight lesions on the other hand, become relatively very extensive without any coalescence whatever. It is not improbable that the description of the old spots refers to the latter type of lesion, since fructifications of *Helminthosporium turcicum* are always visible as a grayish efflorescence in the central portion of large affected regions. In the material from both Florida and the Philippines as obtained from the field, the fructifications on the leafspot lesions have been too sparse to be macroscopically visible. Since, however, on incubation under moist conditions a copious production of sporulating fructifications ensues, it might be wrong to assume that the "black mold" described by Marquez could not possibly have been produced by the leafspot parasite. The total of microscopic detail given by Marquez is equally difficult to reconcile with the morphology of either species alone. From 20 measurements of conidiophores he found these structures to vary from 96 to $148\ \mu$ in length and from

8 to 10 μ in diameter; while 200 spores varied from 36 to 112 μ in length, and from 10 to 18 μ in diameter. As the sporophores and spores of *H. turcicum* do not differ greatly in length from those of the leafspot fungus, the figures given for this dimension might be nearly equally acceptable for either species. On the other hand, the diameter of these structures differs considerably between the two parasites. The range given for the diameter of conidiophores would seem much more acceptable for *H. turcicum* than for the fungus causing leafspot; while the range in spore diameter is certainly characteristic of the leafspot parasite and not at all characteristic of *H. turcicum*. Marquez' statement that "the conidia are wider at the middle and gradually taper toward the two ends; they are septate, and are crescent-shaped" would seem to lend additional support to the assumption that he dealt with the leafspot fungus.

The inferences drawn from the publications of Reinking and of Marquez are altogether in harmony with direct evidence provided by examination of various lots of diseased material originating in the Philippines, mostly in the region in which those writers made their observations. The specimens of affected maize leaves collected near Los Baños in November, 1921, to which reference has already been made, exhibited a number of lesions typical of leaf blight, some of them 15 and 20 cm. long, with an abundance of fructifications in the center of the dead areas. The spores obtained from such areas differed in no wise from the conidia associated with leaf blight encountered in the Middle Atlantic States—broad, mostly straight, tapering markedly toward the basal end, provided with a protruding hilum, and with septa not exceeding eight in number. In addition, the leaves bore a much larger number of spots altogether similar to those present on the Florida material—similar in color and zonation as well as in being delimited laterally, when well developed, by the larger veins, which as the specimens represented more mature foliage, were spaced at intervals of approximately 3.0 mm. Preparations of the discolored areas revealed a relatively sparse development of conidiophores and conidia, which after treatment with chloral hydrate showed, except for the degenerate contents and swollen membranes characteristic of dead structures, complete similarity in morphological detail to the Florida fungus as collected in the field. The specific identity of the two forms was established beyond any question when some of the Philippine material was incubated in damp chambers. Some of the spots gave rise to an outgrowth of sporophoric filaments bearing conidia differing in no particular—size, septation, coloration, curvature, or basal modification—from those resulting from the incubation of Florida material under like conditions.

The fresh conidia were utilized in making a number of single-spore cultures, which again were completely like those of the Florida parasite. Complete similarity was found to obtain also in regard to perithecia, asci, and ascospores, as well as to the conidial fructifications developed in cultures derived from ascospores or pieces of immature perithecium. The fact that the material collected in November, 1921, gave rise to fresh fructifications when placed in a damp chamber as late as October, 1924, is indicative of a considerable measure of longevity in the mycelium of the fungus found within the tissues of the diseased leaves, since the old conidiophores and conidia could in no instance be made to germinate.

A specimen deposited in the herbarium of the Office of Pathological Collections bears the following label:

Philippine Fungi, Herbarium, College of Agriculture, Los Baños, Philippines No. 5. *Helminthosporium inconspicuum*. On leaf of *Zea mays* Linn. Los Baños. Laguna, P. I. Date, Dec. 17, 1919. Coll. L. Goco. Det. by O. A. Reinking.

This specimen was found to consist of a portion of maize leaf bearing numerous well-characterized leafspot lesions. A larger discolored region was also present which may have been a leaf-blight lesion, but if so, it had not developed far enough to exhibit the fructifications of *H. turcicum*.

In four collections of material made by W. H. Weston, jr., in the vicinity of Los Baños on July 17, 1918, September 10, 1918, September 18, 1918, and January, 1920, the injury observed could be assigned without difficulty and presumably with certainty, to the parasite under discussion, either alone or together with *Helminthosporium turcicum*. The first of the collections made in a field where three-fourths of the stand had been killed reveals both leafspot and leaf-blight lesions, the former scattered profusely everywhere over the foliar organs, the latter relatively few in number, but frequently very extensive, and often including numbers of leafspot lesions within their boundaries. The second collection made in a test plot where infection was described as rather serious, appeared entirely free of leaf blight, but exhibited an abundance of leafspot lesions. In the third collection, which was made in a field showing "noticeable loss," most of the leaves bore only leafspot lesions, while others were affected with leaf blight as well. On the young foliage that constitutes the collection of January, 1920, only leafspot lesions are evident, these being, however, both numerous and well developed. Although no general conclusions can be based on the few collections examined, it would appear that in 1918, 1919, 1920, and 1921 the leafspot disease occurred abundantly in the Philippines, manifestly being responsible for moderate losses, even in the absence of leaf blight, and, where combined with the latter malady, responsible apparently in large part for instances of more destructive injury.

In a brief discussion of Mitra's interesting paper on the disease of maize and sorghum attributable to *Helminthosporium turcicum* in India (8), attention was called to certain discrepancies in morphological detail between the account of the parasite given by that author and the account submitted by the writer from studies made in the Middle Atlantic States. It is believed that a partial explanation of these discrepancies may be found in the assumption that Mitra was dealing not only with the leaf-blight parasite, but with the leafspot fungus as well, notably in the Pusa and Malda specimens, which yielded spores described as "curved and narrow." Certainly some of the figures of spores from the Pusa maize leaves (8, pl. II, 9, 10, 11, 13) are more suggestive of the leafspot fungus than of *H. turcicum*, while the others (8, pl. II, 7, 8, 12) would seem to be more aptly illustrative of *H. turcicum*. It must be admitted, to be sure, that Mitra's statement that the lesions on the Pusa and Malda material were similar to those on specimens from other localities, and that cultures derived from this material bore spindle-shaped, straight, or rarely curved conidia does not lend support to such an explanation.

In the foregoing, attention has been confined to leafspot and leaf blight of maize, and the congeneric causal organisms responsible for them, without reference to the occurrence of *Helminthosporium* infection of the male inflorescence of maize, mentioned in several publications based on studies in the Tropics. Reinking, in his discussion of *H. inconspicuum* (10), states that: "A black mold is produced on the tassel * * *. *Helminthosporium curvulum* Sacc. has been reported from the tassels of corn." In another paper (11) the same matter is restated as follows: "A black mold is also produced on the tassel. Saccardo has determined this fungus as *Helminthosporium curvulum* Sacc.; however, it produces a disease identical with the earlier described disease caused by *Helminthosporium inconspicuum*." In a host index of Philippine economic fungi, *H. curvulum* is enumerated among the fungi on corn as "tassel mold."

Mitra, in his description of maize blight, states that—

In the male inflorescence the disease assumes a blackish mold-like appearance on the surface of the glumes of male spikelets. The attack is not extensive and scattered spikelets here and there are infected. The mycelium ramifies within the tissues of the paleae and the stamens.

* * * Conidia of the fungus from the glumes of male spikelets are a little longer and more curved than those of the leaf fungus and has been named *H. curvulum* Sacc. In the Philippines it is described by this name but here in cultures it resembles the strain from the leaf and, when inoculated on the leaves, produces the same kind of spots as the leaf strain.

To these references may be added a passage from the writer's account (2, p. 716) of *Helminthosporium turcicum*, regarding the conidia obtained from affected maize tassels collected in the Philippines by W. H. Weston, jr.:

Preparations made from the fructifications on the tassel, however, showed conidia which, while of the same color and approximate maximum length, were perceptibly inferior in diameter, measuring approximately 11 to 14 μ in this dimension; more abundantly septate, 12 transverse walls being not uncommon; usually quite distinctly curved; and evidently similar to those figured by Reinking (10, pl. 20, B, C). * * * If the forms on the leaves and on the tassels should indeed prove to be identical, the morphology of *Helminthosporium turcicum* as generally understood would stand in considerable need of revision.

Subsequent to the discovery of the leaf-spot disease, the writer took occasion to examine two packets of affected maize tassels deposited in the herbarium of the Office of Pathological Collections by C. F. Baker under the label:

"*Fungi malayana* No. 239 *Helminthosporium curvulum* Sacc. n. sp. on *Zea mays*. Mount Maquiling, near Los Baños, Province Laguna, Philippines. Det. by Saccardo. Date Aug., 1914."

In general appearance the specimens closely resembled those collected by Weston, the fructifications where best developed being crowded on the scales as a velvety layer (fig. 3, F) very similar to the growth of *Helminthosporium oryzae* B. de H. on the inflorescence of rice. Microscopic examination revealed two species of *Helminthosporium*, one with relatively small sporophores (4 to 6 by 100 to 225 μ) and small 3-to-4 septate, curved conidia, measuring 9 to 14 μ in diameter by 20 to 30 μ in length, the middle cell of which was considerably smaller and darker than the other segments (fig. 3 Ba to Bf, Ca to Cm). The other fungus was apparently identical with the one found on Weston's material, the conidiophores measuring usually from 4.5

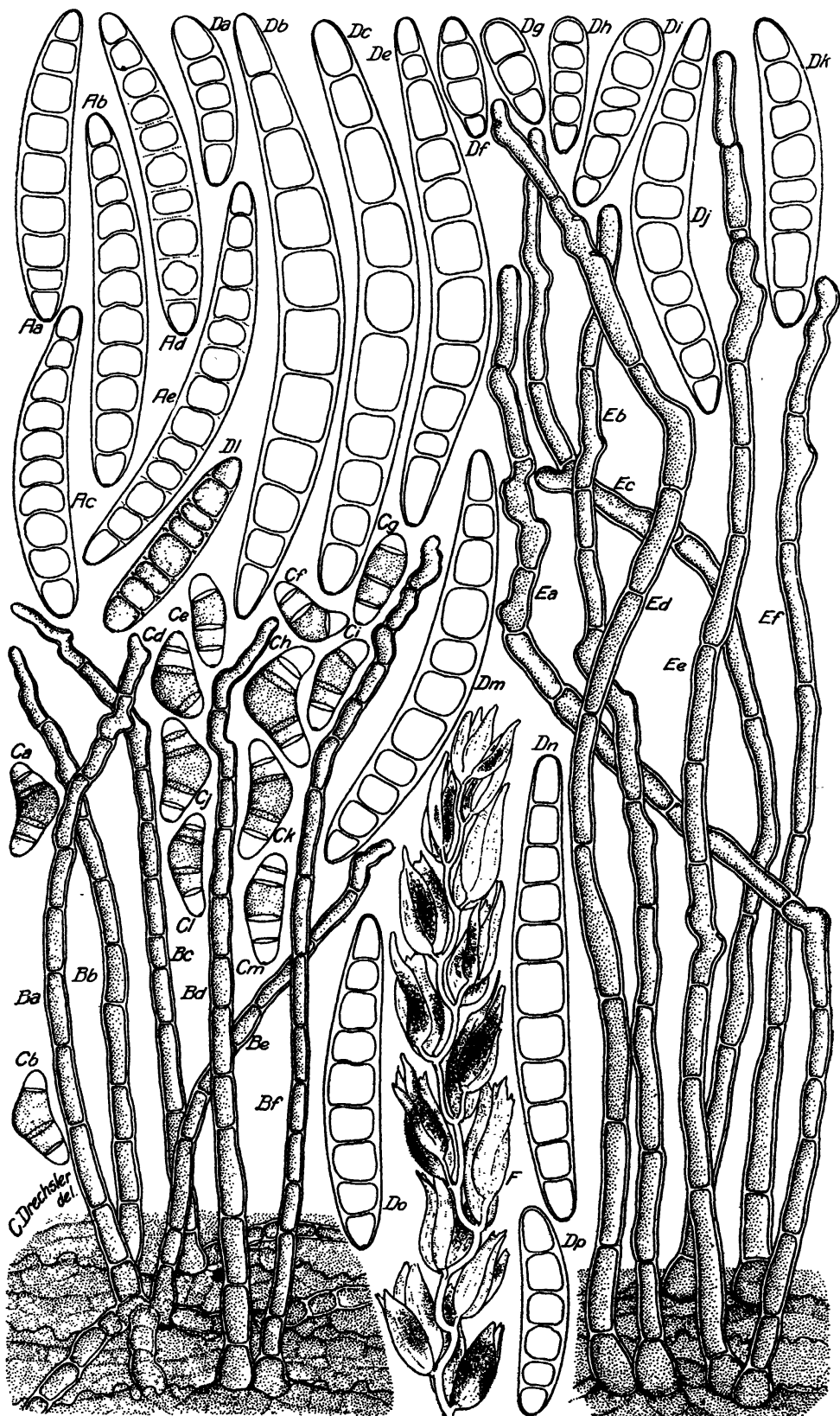


FIG. 3.—Aa–Ae. Conidia of *Ophiobolus heterostrophus* from specimen of maize leaf collected at Brooksville, Fla., June, 1917, deposited in herbarium of the Office of Pathological Collections. $\times 500$

Ba–Bf. Conidiophores of the smaller species of *Helminthosporium* present on the specimen of maize tassels distributed as “Fungi Malayana No. 239,” and evidently the one which Saccardo identified as *H. curvulum* Sacc. $\times 500$

Ca–Cm. Conidia of the same species. $\times 500$

Da–Dp. Conidia of the larger tassel mold present in the specimens distributed as “Fungi Malayana No. 239,” after treatment with chloral hydrate; not *H. curvulum*, nor *H. turcicum*, though perhaps *Ophiobolus heterostrophus*. $\times 500$

Ea–Ef. Conidiophores of the foregoing species. $\times 500$

F. Portion of infected tassel from “Fungi Malayana No. 239” bearing a mixture of two species of *Helminthosporium*. $\times 0.1$

to $8.0\ \mu$ in diameter, and sometimes more than $340\ \mu$ in length (fig. 3 *Ea to Ef*), while the light olivaceous, generally curved 2 to 12 septate conidia, gave as the more extreme measurements, 10 to $17\ \mu$ for diameter, and 28 to $155\ \mu$ for length (fig. 3, *Da to Dp*). When treated with chloral hydrate, the hilum appeared as a broad, rather inconspicuous scar included within the contour of the basal cell, not as a protruding modification. It was in all probability the latter fungus which Reinking and Mitra regarded as the leaf-blight fungus, and which apparently they believed Saccardo had identified as *H. curvulum*.

As to the correctness of a view holding the larger tassel mold identical with the leaf-blight fungus, no final decision is possible without a comparison of living material of both forms developed under similar conditions. In the absence of cultures of the larger species obtained from diseased tassels, the writer has been unable to make such comparison. He is, nevertheless, quite convinced that the two forms represent altogether distinct species—that, in short, the larger tassel mold is not *Helminthosporium turcicum*. The possibility remains, of course, that *H. turcicum* may occur on male inflorescences of maize to some extent, although the writer has not observed anything he could have identified with this species on any of the specimens examined by him.

In comparing the larger tassel mold with the leafspot fungus, a striking degree of similarity at once appears. In respect to diameter and spacing of septa in conidiophore and conidium, the two forms agree closely. Both are similar in that the conidia are strongly curved, are septate up to twelve times, are from 10 to $17\ \mu$ in diameter, and have a broad scar contained within the contour of the basal cell. The only evident difference is the length of conidiophore and conidia, these dimensions attaining considerably greater maxima in the material from affected tassels, although the difference between the general run of spores is not especially marked. It is possible that a condition prevails here similar to that found in the case of *Helminthosporium oryzae*, which produces, on the mats of well-developed sporophoric filaments that constitute the black mold on the inflorescence, conidia considerably longer than those produced on the poorly developed sporophores arising sparsely from the foliar lesions. Although the writer is inclined to regard the larger tassel mold on maize as identical with the parasite causing maize leafspot, he is not prepared to offer this view as a conclusive opinion.

In any case it appears certain that Reinking and Mitra were in error in assuming that the tassel mold which they regarded as identical with *Helminthosporium turcicum* was the one which Saccardo identified as *H. curvulum* Sacc., in spite of the aptness of the latter specific name. As the diagnosis of *H. curvulum* (14, p. 89) did not appear until 1919, Reinking probably drew a natural but mistaken inference from an examination of Baker's Fungi Malayana No. 239. The species is based apparently on a fungus on leaves of *Bambusa blumeana*, and in part is characterized as follows:

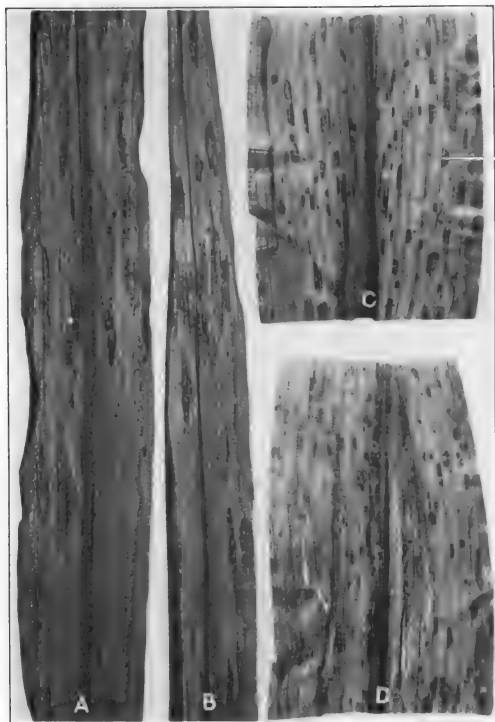
* * * Conidiophoris dense fasciculatis, filiformibus, $90-100=6-7\ \mu$, septatis, fuliginis, sursam pallidioribus obsolete denticulatis; conidiis obclavatis, distincte curvato-gibbis, $35-40=9-12$, utrinque, praecipae basi rotundatis 3-septatis, non constrictis, grosse 4-guttatis, fuliginis, loculis binis mediis obscurioribus.

Unquestionably Saccardo's identification was concerned with the smaller species of *Helminthosporium* present on Baker's specimens as tassel mold, one of the several types frequently encountered when dead grass leaves are incubated in a damp chamber. In spite of its general distribution in nature, it appears to be of relatively little significance in relation to disease, and is discussed here only to clear up nomenclatorial confusion.

OCCURRENCE ON TEOSINTE

With the several lots of diseased maize leaves kindly sent by Weston were two liberal lots of teosinte (*Euchlaena mexicana* Schrad.) leaves collected in September and November, 1918, in the same locality as the maize collections. These leaves exhibit lesions strikingly similar to leafspot lesions on maize in shape and coloration, though minor differences are apparent in some instances in the less regularly straight lateral margins and the somewhat greater length of the lesions on teosinte, which may measure up to 3 cm. in a longitudinal direction (pl. 1, A, B). The lateral edges, in general, did not coincide as closely with the veins of the host as on maize. Microscopic examination revealed in the central parts of the dead areas, a sparse production of conidiophores and conidia, which when treated with chloral hydrate showed complete similarity to those on maize. Owing evidently to application of preservatives, efforts to recover the parasite in a living condition by incubating infected material in a damp chamber did not succeed. However, because of the close similarity in morphology and pathological habit, there can be little doubt that the same parasite is involved in the foliar disease of the two closely related hosts, which, it may be mentioned, have several other fungous parasites in common.

The occurrence of the leafspot fungus on teosinte raises the question as to the probable identity of the parasite found in the United States and in the Philippines with *Helminthosporium euchlaenae* described by Zimmerman (17) as pathogenic to this host in German East Africa. Zimmerman's description of the foliar lesions produced by the African form as light-brown spots with dark-brown margin, mostly elongated in a longitudinal direction, approximately 2 mm. wide and up to 25 mm. long, applies unusually well to the Philippine material. A not unsatisfactory agreement prevails also with reference to the sporophores which, according to the German account, are brown, unbranched, several times septate, 5 to 7 μ in diameter and up to 150 μ in length, and emerge from the stomata singly or in groups of two or three. The characterization of the conidia as brownish, cylindrical, straight or somewhat curved, up to seven times septate, 50 to 60 μ long and 13 to 15 μ in diameter, would seem to represent these structures as considerably inferior in degree of curvature, frequency of septation, and maximum length to the conidia on the Philippine specimens. If Zimmerman's text and figures give a sufficient description of the conidia of the African fungus, it would be necessary to conclude that the latter constitutes a species distinct from the Philippine form. However, with a fungus sporulating as sparingly as the leafspot parasite, the possibility of a description being based on a small number of conidia not altogether characteristic of the species is far from being a remote one. The question concerning the identity of the leafspot parasite with *H.*



A, B.—Portions of teosinte leaves showing lesions of *Ophiobolus heterostrophus*. Photographed from dried specimens originating from the vicinity of Los Baños, P. I., September, 1918.

C.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a much larger lesion caused by *Helminthosporium turcicum* on the upper left margin. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. $\times \frac{3}{4}$

D.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a part of two moderately extensive lesions caused by *H. turcicum*, one immediately adjacent and to the right of the midrib, the other toward the left margin. The border of the second lesion is indicated by a dotted line for greater clarity. The cracks due to the brittle texture of tissues killed by *H. turcicum* indicate approximately the center of the leaf-blight lesion. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. $\times \frac{3}{4}$

euchlaenae therefore can not be decided until additional information on the African form becomes available. It may be added that Zimmerman makes no mention of any leafspot on maize, although his account of maize rust makes it evident that this host came under observation.

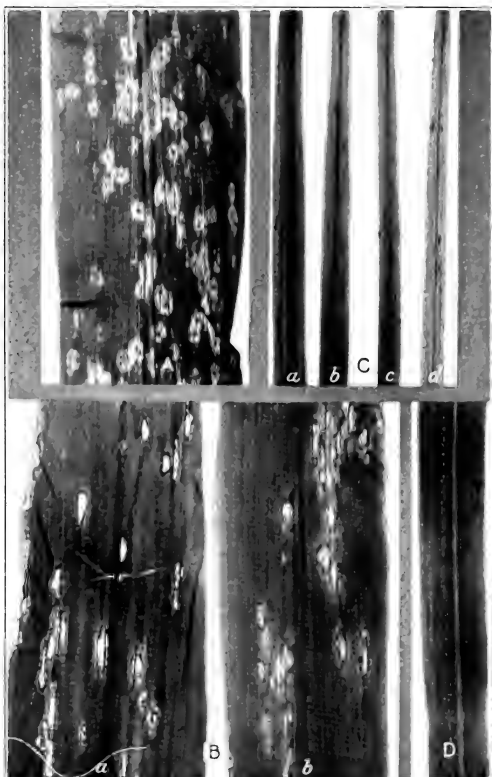
INOCULATIONS ON RICE AND SUGAR CANE

Morphologically, the parasite causing leafspot of maize shows much similarity to *Helminthosporium oryzae*, *H. sacchari* Butler, and *H. leersii* Atk. Perhaps its most obvious distinctive characteristic is found in the curvature of the conidium, this curvature being more pronounced than in any large-spored congeneric form known to the writer. The conidia of both *H. sacchari* and *H. leersii* would appear to be somewhat inferior in diameter to those of the maize parasite. Owing to a considerable degree of variability manifested by species of *Helminthosporium* under different conditions, it was believed advisable to determine the possible pathogenicity of the Florida organism on rice (*Oryza sativa* L.) and sugar cane (*Saccharum officinarum* L.), since these hosts are cultivated in tropical and subtropical regions where leafspot of maize is presumably of fairly widespread occurrence.

For the inoculation tests, plants grown in 5-inch pots in the greenhouse were used. The experimental material consisted of 24 pots each of sweet corn, dent corn, rice, and sugar cane, the maize being planted 3 seeds to a pot, the rice 10 seeds to a pot, and the cane started with a single cutting to each pot. Inoculations were begun 25 days after planting, when all the pots showed very satisfactory growth, and were repeated five times, at intervals of four days, the last material used thus being 45 days old, and in the case of the maize and cane, so large as to be somewhat unwieldy. Three pots of each host were inoculated at a time, by applying with an atomizer a suspension of conidia derived from pure cultures of the leafspot fungus on plates of corn-meal agar. Immediately after inoculation the plants, together with an unsprayed pot of each host as control, were incubated in a large moist chamber provided with glass windows.

On examining the maize plants 36 hours after inoculation, most of the leaves were found to be covered with hundreds of watersoaked spots varying in diameter between 1.0 and 2.5 mm., with the center occupied by a very minute whitish speck (pl. 2, A). The control plant immediately after this period showed no ill effects from confinement in moist atmosphere, but at the end of 48 hours showed symptoms of etiolation which became increasingly pronounced as incubation was continued to 72 or 96 hours. Even after 96 hours all except the lowest foliage of the controls was still functional, whereas the leaves of the inoculated plants had utterly collapsed and were converted into wet, softened structures drooping flabbily from the stalks.

An apparently more natural course of development of the infection resulted when the experimental plants were removed from the damp chamber to the greenhouse 24 or 36 hours after inoculation. Portions of the leaves in which the infections were very numerous withered very soon, to be sure, the healthy parts dying evidently from an interruption of the water supply, but where a more moderate



A.—Maize leaf 42 hours after inoculation by spraying with conidial suspension of *Ophiobolus heterostrophus*. Photographed by transmitted light, the light areas corresponding to watersoaked spots. $\times 3/2$

Ba-Bb.—Portions of maize leaf inoculated with conidial suspension kept in damp chamber 36 hours, then in greenhouse 15 days. $\times 3/2$

Ca-Cd.—Portions of rice leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not subsequently increasing in size. $\times 3/2$

D.—Portion of sugar-cane leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not increasing in size. $\times 3/2$

distribution prevailed the tissues withered only in the regions directly involved in the lesions. The enlargement of the latter took place especially toward the base and the tip of the leaf, lateral extension being in most instances, though not in all, checked by the veins. This elongation resulted in the characteristic lesions found on the original specimens—a striplike spot of uniform width, variegated with delicate, reddish-brown, elliptical or transverse zonation, and surrounded by a narrow reddish-brown marginal zone. The zonation, as expected, was found to have its origin in the intermittent extension of the lesion. During periods of high humidity prevailing often as a result of watering or the lowering of temperature during the night, a zone of healthy tissue immediately below and above the established lesion became watersoaked in appearance. With the recurrence of dry conditions, these portions of tissue withered and appeared as additional zonal increments (pl. 2, B, *a* to B, *b*).

In contrast to the heavy infection brought about on both field corn and sweet corn, most of the rice plants subjected to the same treatment showed no evidence of even incipient lesions. On two of the lots a sparse scattering of minute blackish specks appeared 36 hours after being inoculated. As the rice plants were found to flourish in the damp chambers, better indeed than in the greenhouse, the pots which had yielded the incipient infections were retained in the chamber for 20 days. The spots, however, increased neither in size nor in number, and at the time the material was discarded the foliage was in thriving condition (pl. 2, C, *a* to C, *d*).

The sugar cane responded to the inoculations in a manner somewhat similar to rice. Nearly all the cane plants developed minute, dark, rather indefinitely delimited specks on the foliage 36 hours after being sprayed with the suspension of conidia. As they appeared to suffer no ill effects from being exposed to the humid conditions of the damp chamber they were retained in the chamber for more than 20 days. None of the lesions, however, evidenced any tendency toward further development (pl. 2, D).

The vigorous parasitism of the fungus on maize foliage contrasts strongly with its failure to produce anything but incipient infection, in spite of especially favorable conditions for development, on the leaves of rice or sugar cane. That the organism would infect rice or sugar cane under natural conditions appears highly improbable. Its biological behavior is thus confirmatory of morphological evidence indicating its specific independence of *Helminthosporium oryzae* and *H. sacchari*.

ASCIGEROUS STAGE OF THE PARASITE

¶ In discussing the specific identity of the fungus strains developing on the diseased maize leaves from Florida and from the Philippines, mention was made of their association with entirely similar ascigerous fructifications. The latter develop side by side with the conidiophores when diseased maize leaves are incubated in a damp chamber, although the minute black masses that constitute the young perithecia can not usually be discerned with the naked eye until the third or fourth day. Apparently the initial stages of development take place under the epidermis, which, however, is generally soon broken through and the sclerotiumlike bodies exposed to view. In its later stages

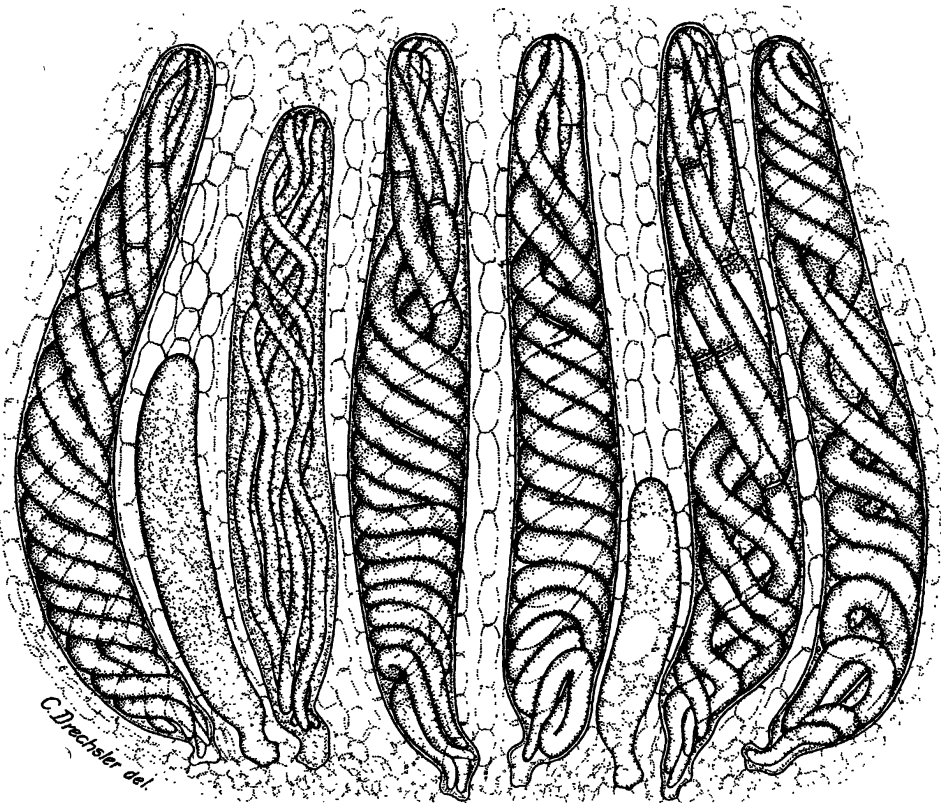
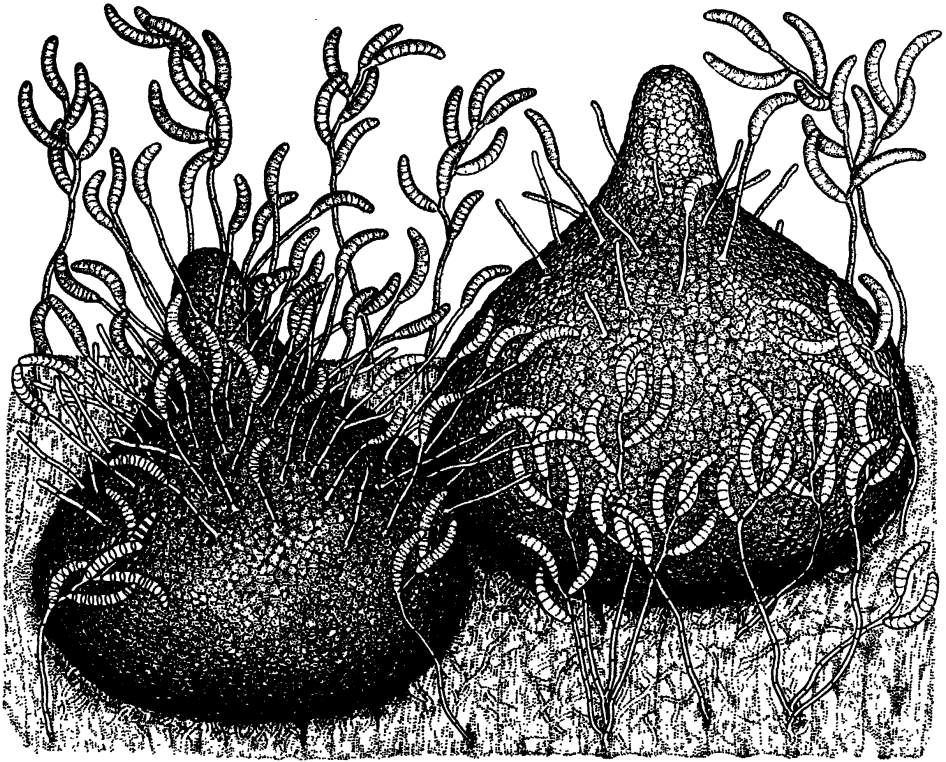


FIG. 4.—A (upper). Two mature perithecia of *Ophiobolus heterostrophus* on diseased maize leaves incubated 20 days in a damp chamber, illustrating some variation in size and shape, and relationship to conoidal fructifications. $\times 120$
 B (lower). Asci in various stages of maturity together with vertically oriented filaments that compose the colorless pseudoparenchyma in the interior of the perithecium, which diminishes with the development of the asci. $\times 473$

the fruiting body has the appearance of a superficial structure (fig. 4, A, upper). In some instances the rupturing of the disintegrating host tissues is delayed longer, while in the case of perithecia developing on the filter paper over which the fungus spreads from infected material, even the earlier stages are practically superficial, the loose mat of overlying fibers scarcely affording any substantial covering. The globose portion of the perithecium attains its definitive size in about 6 to 10 days, and a few additional days are usually required for the beak to reach its final proportions. Externally the fruiting body is black and relatively smooth, though often beset toward the base with stubby cylindrical projections resembling incipient conidiophores, or, perhaps, the proximal part of fractured conidiophores. The dark pseudoparenchymatous external layer is relatively thin, while the interior consists at first of firm colorless pseudoparenchyma composed of laterally appressed, vertically oriented filamentous elements. Young asci can generally be made out here and there after 12 days, and at the end of 15 days are not at all rare. As the asci increase in size and number the pseudoparenchymatous structures diminish correspondingly. Perithecia from 18 to 20 days old generally yield, on being crushed, a small proportion of approximately mature asci; the condition illustrated in Figure 4, B (lower), where most of the asci are mature, being, however, not attained usually in less than 25 or 30 days.

The mature asci, except for the short, markedly contracted stipe, are roughly cylindrical in shape, though generally widest about one-third of their length from the base, from which point the diminution in diameter toward the apex is more gradual than toward the base. When crushed out individually they frequently show more pronounced distention, evidently as a result of the imbibition of water preliminary to dehiscence, when such imbibition takes place in the absence of pressure normally provided by the surrounding asci and filamentous elements. Those in the center of the fruiting body are usually straight, while others near the periphery are distinctly curved, to conform with the curvature of the subspherical perithecial wall (fig. 5, A). Each ascus contains typically four cylindrical ascospores coiled in a close helix, each spore usually exhibiting approximately four turns, which alternate regularly with the turns of the other spores. As the lower ends of one or more spores thrust well into the short contracted stipe the helicoid arrangement at the base of the ascus is constrained into some irregularity. The upper ends of the spores similarly thrust into the apex of the ascus, yet, owing apparently to the more regular conformation of the apical portion, the departure from the spiral arrangement is usually very slight. In several hundred asci examined the rotation of the helix has been found to be always in the same direction; that is, on an upper focus with the ascus in a horizontal plane following the spore from the base toward the apex the coil appears at right and disappears at the left. The four spores might thus be compared to the threads of a quadruple left-handed screw.

The earlier stages in the development of the ascospores are not easy to make out, at least in unstained preparations, as the young structures are distinguished in the granular protoplasm only with difficulty. The spores first become clearly visible in the still growing ascus as filamentous bodies; approximately 3 μ in diameter and about

125 μ in length, consequently extending nearly the entire length of the ascus within which they follow an irregularly sinuous course. Subsequent growth ensues both in length and diameter, so that the final dimensions are frequently more than double those indicated. The length of the spores thus comes to exceed that of the ascus, a condition which is made possible by the helicoid coiling of the ascospores, the turns of which become increasingly close as maturation proceeds. After a rather tight arrangement has been effected, cross walls make their appearance, beginning at the upper end of the spore and proceeding downward.

When a perithecium containing mature asci is crushed in a water mount, a brisk discharge of ascospores ensues. As might be expected because of their involved arrangement, the spores of each ascus are discharged simultaneously, the discharge always taking place from the apex, and with enough violence to propel the spores a short distance through the water, the helicoid coils thereupon slowly becoming relaxed in loose, sinuous curves. Judging from the instances in which dehiscence is impeded, frequently as the result of immature condition, discharge is preceded by some swelling of the ascus (fig. 5, B, C, D), and circumscissile rupture in the apical portion of the ascus wall (fig. 5, E). Intertwined spores in groups of four have occasionally been observed adhering to the tip of the beak of perithecia examined in place, leaving little doubt that normally the spores in each ascus are discharged through the ostiole simultaneously as a group.

On being liberated, either naturally or by manipulation, the ascospores appear as filamentous structures distinctly fuliginous in coloration, tapering somewhat at the extremities (fig. 5, H, K). The constrictions at the septa which previously were not pronounced usually become more clearly accentuated, evidently as a result of some slight enlargement of the delimited segments. The contents are somewhat granular, especially immediately adjacent to the septa. In some instances a mucous envelope is apparent (fig. 5, L, M). When properly mounted in water the spores germinate very promptly by the production usually of one to eight germ tubes which may originate terminally from the tip or laterally from any part of the spore (fig. 5, F, G).

It should be mentioned that while the number of spores in an ascus is typically four, cases in which only three are present are by no means rare, and even one-spored and two-spored asci may be encountered occasionally. In the latter instances the asci are less completely occupied, the helicoid turns are looser and more irregular, although the direction of rotation remains the same. In cases of frustrated dehiscence, where the spores are only partly liberated from the ascus wall, germination is not materially impeded, the germ tubes arising from the lower segments of the ascospores perforating the surrounding membrane without any apparent difficulty.

Cultures made by placing fresh asci on corn-meal agar plates and transferring the resulting growth to sterile agar slants, show the same type of growth and produce the same type of conidiophores and conidia as are found on cultures originating from conidia. Conidia obtained from such cultures when applied to maize foliage have been found to give rise to lesions indistinguishable from the lesions resulting from the use of conidia from other sources. The facts regarding

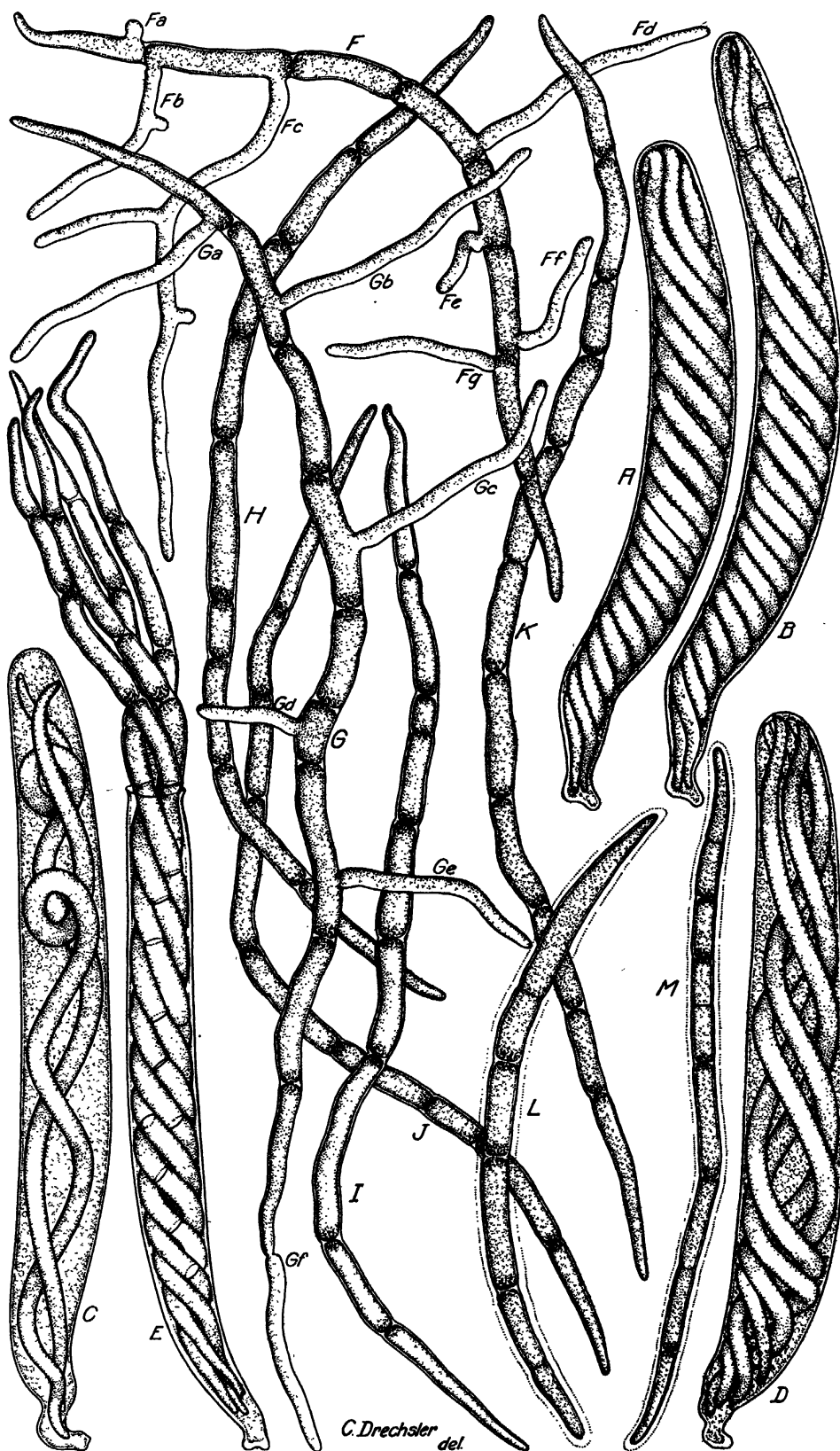


FIG. 5.—A. A mature or nearly mature ascus of *Ophiobolus heterostrophus* as obtained from a freshly crushed perithecium. $\times 476$
 B. The same ascus as in A, an hour later, showing elongation presumably preliminary to dehiscence; and the insertion of septa in the apical portion of the spores
 C, D. Asci like B, after elongation, C containing only 2 spores. $\times 476$
 E. Partial liberation of ascospores by rupture of ascus toward apex; evidently an instance of frustrated discharge. $\times 476$
 F, G. Ascospores germinating by production of germ tubes Fa-g, Ga-f, respectively. $\times 476$
 H, I, J, K. Ascospores in mature septate condition. $\times 476$
 L, M. Mature ascospores inclosed in a mucous sheath. $\times 476$

culture and pathogenicity only serve to confirm, however, the very clear evidence provided by the frequent presence on the perithecium of a larger or smaller number of conidiophores bearing conidia typical of the leafspot fungus and arising unmistakably from the perithecial wall (fig. 4, A). Sometimes the conidial fructifications arising in this manner become very abundant, with the usual result that the internal differentiation of the fruiting body is largely if not entirely suppressed.

CLASSIFICATION OF PARASITE

In reference to Saccardo's system, the fungus is presumably to be reckoned among the scolecosporous Sphaeriaceae, by virtue of the relative dimensions of the ascospores rather than because of any close similarity to the usual filamentous type. Among the definitions of the genera included in this category, none contain any special provision for the most noteworthy characteristic of the fungus—the disposition of the ascospore in a well-defined spiral. A considerable degree of similarity is discernible, however, to a fungus originally described by Saccardo (12) as *Rhaphidophora camptospora*, a combination later reduced by the author (13, v. 2, p. 344) to a synonym of *Ophiobolus camptosporus* Sacc. Under the latter binomial, Berlese published illustrations (1, v. 2, pl. 159) and a somewhat altered description (1, v. 2, p. 133) based upon material supplied by Saccardo. Berlese's figures show a short, stipitate, cylindrical ascus containing four spores partly coiled in helices in a manner strongly suggestive of the maize parasite, although the direction of rotation is opposite to that prevailing in the latter. In *Ophiobolus camptosporus* one of the median segments in the ascospores is swollen, which would seem to show relationship to certain other forms assigned to *Ophiobolus*, as well as to species referred to allied genera exhibiting one or more nodose intermediate segments.

Another fungus that should be mentioned in this connection is *Ophiobolus chaetophorus* (Crouan) Sacc., which, according to the account given by Malbranche and Niel (6), exhibits a spiral disposition of its ascospores suggesting the elaters of certain slime molds. In the figures, the direction of rotation seems to be like that found in the maize parasite. As the asci are described as eight-spored, and the perithecia as provided with rigid bristles on the upper part, differences from the latter organism are not lacking. *O. helicosporus* (B. & Br.) Sacc. (13, v. 2, p. 350) with "sporidiis linearibus praelongis spiraliter convolutis" and *O. galii* Rich. with "sporidiis spiraliter fasciculatis, hyalinis" represent two imperfectly described species that may possibly be related. Malbranche and Niel state that the perithecia of *O. helicosporus* are glabrous, thus differing in one particular from *O. chaetophorus*, which Saccardo later referred to the genus *Ophiochaete*, erected to include definitely setose forms.

According to recent publications by Höhnelt (4, 5) and Weese (16), the genus *Ophiobolus*, as employed by Saccardo and other writers, is far from being a homogeneous group. Höhnelt's recommendations contemplated the maintenance of three genera—(1) *Leptospora* Rabh., based on *L. porphyrogona* (Tode) Rabh. to include not only "sphaeriaceous" forms having narrow spores with or without a nodose cell, but also species having somewhat broader many-septate, colored spores with nodose segments; (2) *Entodesmium* Riess, based on *Entodesmium rude* Riess, a "dothidiaceous" type, to include those

species the spores of which become disarticulated within the ascus; and (3) *Ophiobolus* Riess, established on *O. acuminatus* (Sow.) Duby to include "sphaeriaceous" forms with nodose spores, presumably broader than those referable to *Leptospora*. An additional and new genus, *Leptosporopsis*, was proposed for certain other forms like *Ophiobolus rostrupii* Ferd. and Winge, *O. compressus* Rehm and *O. tanacetii* (Fuck.) Sacc. which Höhnelt found to possess "dothi-disceous" structure and hence to differ from *Leptosphaeria* de Not. only in having long narrow spores. Weese adopted Höhnelt's revision of the group, suggesting, however, changing the definitions of the closely related "sphaeriaceous" genera *Leptospora* and *Ophiobolus*, so that the former is to include only forms with filamentous spores without nodose segments, while the latter is to include the species with nodose spores. For the setose forms, Weese retained two genera, Saccardo's *Ophiochaete*, to include forms with erumpent fruiting bodies analogous to *Ophiobolus*, and *Acanthophiobolus* Berlese to include species with superficial perithecia and worm-shaped, multiguttulate, subhyaline ascospores. Since the latter combination of characteristics was found represented in *Sphaeria chaetophorus* Crouan, the retention of the combination *Acanthophiobolus chaetophorus* (Crouan) Berlese, abandoned by its author in favor of Saccardo's combination, was indicated.

Although the taxonomy promoted by Höhnelt and Weese may redistribute an assortment of fungi into a number of presumably less heterogeneous groups, it is not certain that the rearrangement proposed is, after all, as free of arbitrary features as might be desired. The distinction drawn between the "sphaeriaceous" and "dothi-disceous" types of discrete perithecia, on the basis of differences in internal development and structure, is one that has not hitherto been widely incorporated in mycological literature. In any case, the fungus under consideration would appear not to qualify for inclusion in *Entodesmium* as interpreted by Höhnelt, as its spores show no tendency toward becoming disjointed. Nor could it well be referred to *Leptospora*, as the type for this genus, with its extremely slender spores, obviously does not represent a closely related form. While it must be admitted that the type of *Ophiobolus*, *O. acuminatus* (= *Leptosphaeriopsis acuminata* [Sow.] Berlese), with its asci containing eight straight spores, each provided with two swollen segments, does not itself show any close similarity, this species, from the information available, is at least not too obviously different from *O. camptosporus* with its ascus containing four helicoid ascospores each having one nodose intermediate cell to obviate the possibility of their being related more closely than by the arbitrary operations of analytical keys.

Judging from Berlese's figures of *Ophiobolus compressus* (1, v. 2, pl. 156) and *Ceuthocarpon brunellae* (Ell. and Ev.) Berl. (= *Leucospora brunellae* Ell. and Ev.) (1, v. 2, pl. 170), both of which Höhnelt would place in his proposed genus *Leptosporopsis*, these fungi exhibit a degree of similarity to the maize parasite about equal to that shown by the general run of forms referred to *Ophiobolus*, though not as great a similarity as is evidenced by *O. camptosporus*. Indeed, neither *Leptosporopsis* Höhnelt, nor *Ophiomassaria* Jaczewski, based on *O. selenospora* (Otth.) Jacz., a fungus with 2 to 3 septate ascospores in a mucus sheath (13, v. 11, p. 353); nor *Acerbia* as exempli-

fied in *A. therryana*, illustrated by Berlese (1, v. 2, pl. 167); nor *Leptospora* Peng. and Sacc. (13, v. 14, p. 619), would seem to offer any species as similar to the maize parasite as *O. camptosporus*. In the absence of a more appropriate genus to which it could be referred, and in order to conserve for the time being its proximity to the latter form, the leafspot fungus is tentatively assigned to *Ophiobolus*. Such assignment is not intended to imply close relationship to certain species referred to this genus that are frequently mentioned in the literature pertaining to the diseases of cereal crops, as, notably, *O. cariceti* (Berk. and Br.) Sacc., and *O. herpotrichus* (Fr.) Sacc. It may be superfluous to add that the parasite is undoubtedly different from *Acerbia maydis* Rehm, described from dead remains of maize in the Philippines (9), as well as from *Leptosphaeria orthogramma* (B. and C.) Sacc., reported on dead maize culms in Pennsylvania, South Carolina (13, v. 2, p. 60), and the Philippines (9), for in respect to dimensions of spore, for example, the latter forms are decisively inferior to the fungus under consideration.

As far as the writer is aware, the ascigerous stage of the parasite has not been described before. There is scarcely any doubt that the conidial stage has been encountered by pathologists, but with the possible exception of the somewhat problematical *Helminthosporium euschlaenae*, it has apparently not served as type for any species designated by a binomial. As has been pointed out, Saccardo evidently did not intend to apply to it his binomial *H. curvulum*. The combination *H. turcicum* has long been applied, and seemingly altogether correctly, to the fungus causing leaf blight of maize and several other graminaceous hosts. The excessively brief diagnosis of *H. inconspicuum* applies better to the leaf-blight fungus than to the parasite causing leafspot, especially in the details concerning the 3 to 5 septate condition of the conidia and their diameter of 20 μ , although the passage "effused, but so thinly as not to be visible to the naked eye" is not readily reconciled with the actual appearance of *H. turcicum* developing luxuriantly on its host in nature. More direct evidence that *H. inconspicuum* is indeed synonymous with *H. turcicum* is provided by the specimen in the herbarium of the Office of Pathological Collections under the label:

Ellis. North American Fungi. 45. *Helminthosporium inconspicuum* C. & E. Grev. vol. 6, p. 88. On leaves of Indian corn.

Which, judging from the date of issuance, 1878, presumably represents authentic material from approximately the same collection as the type specimens. The conidia present in abundance on the very extensive affected area were found to be altogether of the broad, straight, markedly tapering, relatively sparingly septate type characteristic of *H. turcicum*.

The specific name *heterostrophus*, descriptive of the ascospores, is suggested for the fungus.

DIAGNOSIS

Ophiobolus heterostrophus n. sp.

Occurring on maize (*Zea mays* L.), as the cause of a destructive disease manifested by the appearance on the leaves of numerous dead cinnamon-buff or purplish areas surrounded by a darker reddish brown margin, and often delicately variegated with brownish zonate bands; the lesions longitudinally elongated, first elliptical, later long-rectangular, typically limited to a single intervascular region, usually 1 to 3 by 5 to 15 mm., often coalescing to form more extensive dead portions.

Perithecia developing on disintegrating host tissues, usually early erumpent, black, often bearing a variable number of conidiophores, but no differentiated sterile setae; ascigerous portion subglobose or more frequently somewhat ellipsoidal, measuring usually 0.4 to 0.6 mm. in transverse diameter and approximately 0.4 mm. in vertical diameter; ostiolate beak well defined, subconical or paraboloid, approximately 0.15 mm. at base and 0.15 mm. in length; interior composed of colorless pseudoparenchymatous tissues consisting of vertically oriented appressed filaments, diminishing usually with the development of the asci.

Asci numerous, short-stipitate, with rounded apex, subcylindrical but sometimes becoming more inflated before discharge; 160 to 180 μ in length by 24 to 28 μ in diameter; containing 1 to 4, typically 4, spores. Ascospores filamentous, fuliginous, thin-walled; in somewhat immature condition, of uniform diameter of 6 to 7 μ , except at extremities which are somewhat attenuated; later becoming five to nine times septate, the septa usually associated with perceptible constrictions, the delimited segments becoming somewhat swollen so as to attain in places a diameter up to 9 μ ; thrusting firmly against apex and into base of stipe in multiple heterostrophic helicoid arrangement with approximately four turns to each spore; measuring 130 to 340 μ in length; discharged simultaneously, often with mucous envelope; germinating promptly by producing indiscriminately from any or all segments, either laterally or terminally, germ tubes up to eight in number, from 3.5 to 5.0 μ in diameter.

Conidiophores arising singly or in groups of two or three from stomata in center of killed foliar parts; olivaceous, septate at intervals of 15 to 60 μ ; bearing the first conidium after attaining a length of 50 μ or more; points of attachment of successive spores marked by scars occurring at intervals from 10 to 40 μ at geniculations not always pronounced; in nature measuring usually 120 to 170 μ in length, but under moist condition occurring as irregularly branching sporophoric filaments, often exceeding 1 mm. in length. Conidia developed at 25° C. on diseased maize leaves in damp chambers or in pure culture on artificial media, fuliginous to light olivaceous, measuring 30 to 115 μ in length by 10 to 17 μ in diameter; often strongly curved, usually widest near the middle and tapering perceptibly toward the rounded ends; peripheral wall thin, especially in the apical and basal regions; basal scar broad, not conspicuous, contained within rounded contour; germinating promptly by the production of two polar germ tubes.

Found on diseased leaves of *Zea mays* L. collected at Sanford, Fla., September 22, 1923 (type), Brooksville, Fla., June, 1917 (fig. 3, *Aa* to *Ae*), and at Los Baños, P. I., in 1918, 1919, 1920, and 1921. Perhaps identical with the more luxuriant *Helminthosporium* form widely occurring on the inflorescence of maize in tropical and subtropical regions. Found also on leaves of *Euchlaena mexicana* Schrad. near Los Baños, P. I., September and November, 1918; and possibly to be identified with *H. euchlaenae* Zimm.

TAXONOMIC CONSIDERATIONS

The pleomorphism of the maize parasite casts light on the affinities of a large proportion of the fungi referred to *Helminthosporium*. In their excellent account of *Pleospora polytricha* (15, v. 2, p. 269-271, pl. 29), the Tulasne brothers set forth an association with one representative of the genus which has since been paralleled by the discovery of analogous relationships in other graminicolous forms—*Pyrenophora teres* (Diedicke), *P. tritici-repentis* (Diedicke), and *P. bromi* (Diedicke) found in Europe and America, as well as *Pleospora graminea* Diedicke, the ascigerous stage of the parasite causing the widely distributed stripe disease of barley, which stage, however, has hitherto been reported only from Europe. A significant fact concerning all the species of *Helminthosporium* achieving their perfect form as members of the genera *Pyrenophora* or *Pleospora* is that their conidia are of the straight cylindrical type, germinating by the production of germ tubes laterally or obliquely from intermediate as well as end segments. In pure culture, on many artificial media, under ordinary laboratory conditions, these species are characterized usually by abundant anastomoses in the submerged portion of the mycelium, resulting in the production of complexes of inflated elements plausibly inter-

preted as incipient perithecia, and by conidial apparatus either being absent, or, if present, not infrequently represented by proliferous atypical structures in which sporophoric elements and spores are poorly differentiated.

The maize parasite, like *Helminthosporium sativum* P. B. and K., *H. sacchari* and *H. oryzae*—to mention only a few of the more important species—represents a type of *Helminthosporium* with mostly elliptical, curved conidia, germinating by the production of two germ tubes, both from small, thin-walled regions, one at the apex and the other immediately adjacent to and surrounding the basal scar. In artificial culture, sporulation is usually abundant and not markedly abnormal, although the conidia may be shorter and less regularly curved than those produced under natural conditions. There can be little doubt that the species adhering to this type, including many parasites of higher plants and very probably a considerable number of small-spored forms often referred to the conidial genus *Brachysporium*, are closely related with one another, and that the latter, when connected with ascigerous stages, be found not without similarity to *Ophiobolus heterostrophus*. It is apparent that a proper taxonomic disposition of the general run of species of *Helminthosporium* with bipolar germination is contingent on the discovery of the perfect condition of additional forms, from the morphology of which the common characters may be deduced for incorporation in the definition of a suitable genus, or, if possible, for appropriate revision of a genus already established.

SUMMARY

A foliar disease has been found to occur on maize in Florida and on maize and teosinte in the Philippines, which is characterized by cinnamon-buff lesions which are considerably smaller and much more numerous than those of leaf blight, and are also distinguished by being usually confined to a single intervascular region.

The disease is associated with a fungus which, in its conidial condition, differs from *Helminthosporium turcicum* in the smaller diameter of its conidiophores, as well as in the smaller diameter, more abundant septation, and greater curvature of its elliptical conidia, which, moreover, have an internal basal scar rather than a protruding modification.

The fungus produces discrete, subglobose perithecia with a well defined beak, and bearing asci containing typically four multiseptate, fuliginous, filamentous spores, each coiled in a heterostrophic helix of approximately four turns. It is described tentatively as *Ophiobolus heterostrophus*, n. sp., though not obviously closely related to several well-known species of *Ophiobolus* parasitic on grasses.

The morphological difference between this ascigerous stage and *Pyrenophora* or *Pleospora* lends support to the view that the species of *Helminthosporium* with straight subcylindrical conidia germinating laterally from end and intermediate segments constitute a natural group distinct from the group of species producing curved elliptical conidia germinating by two polar germ tubes.

The leafspot disease is probably widely distributed in tropical and subtropical maize-growing regions, having evidently been confused with leaf blight, which occurs in the same territory. It appears not improbable that one type of tassel mold will prove to be identical with the foliar parasite, in spite of the greater length of its conidia.

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THE TRANSLOCATION OF THE FOOD MATERIALS OF THE WHEAT SEEDLING¹

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INTRODUCTION

At no time in the life history of the plant are the changes due to metabolism proportionally as great as in the seedling stage, when the reserve food of the kernel is changed into simpler and more soluble products and then used as a source of energy or as a part of the young plant. Many of the steps of anabolism and katabolism are poorly understood. Spoehr (37)³ gives a good summary of our present knowledge on this point.

It is the aim of this work to show the chemical and physical changes which take place during the germination of wheat.

Dehérain (11), Harz (22), Teller (38), and Willard and Swanson (45), have analyzed the materials found in the wheat kernel. Thatcher (40), Eckerson (15), and others (25, 39, 2), have followed the formation of these materials in the developing seed. Choate (5) has studied microchemically the changes in the carbohydrate and nitrogen content of seedlings during seven days germination in petri dishes. LeClerc and Breazeale (28), grew seedlings in various types of culture solutions for 15 days, and analyzed the seedlings for the mineral elements, ether extract, reducing and hydrolyzable sugars, pentosans, and fiber. Wimmer (44) studied the various parts of wheat plants, grown in soil, for minerals and starches at various periods, beginning at the time the plants were about 40 cm. high.

METHOD

Spring wheat of the Marquis variety, Clark and Martin (6), was grown in the greenhouse in earthenware containers, 17 by 19 cm., containing soil commonly designated "good field soil." The maximum water-holding capacity of the soil was approximately 50 per cent. The moisture content of the soil was determined and sufficient water added at the time of planting the seeds to bring the content to "optimum." The total weight was then recorded, and water was added frequently to maintain the optimum water content. The plants for the freezing-point determinations were grown in November and December, and those for the chemical determinations in January and February. The temperature of the greenhouse was about 55° F. at night and 70° F. during the day. The plants were collected in the morning before they were exposed to the sun's rays. Since the composition of the plants varies throughout the day and with the conditions varying on different days, as illustrated by the

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² The work represented by this paper was carried on under the direction of A. L. Bakke of the Iowa State College of Agriculture and Mechanic Arts. He suggested the experiment and submitted criticisms at different times, and the writer desires to make acknowledgment of his interest and help. Thanks are also expressed to R. M. Hixon, of the Chemistry Department.

³ Reference is made by number (italic) to "Literature cited," p. 741.

work of Davis, Daish, and Sawyer (9), and Davis and Sawyer (10), it was felt that these variations would be least in the morning. The adhering soil was carefully removed from the roots before they were washed. In order to prevent the drying out of the plants in transferring them from the greenhouse to the laboratory, they were placed in a desiccator which had been converted into a moist chamber.

For the freezing-point determinations, the plants were first dried of adhering moisture by blotting with filter paper, then ground in a mortar until they were reduced to a pulp. This was placed in cheesecloth and squeezed, either by twisting and pressing with pestle or in a tincture press. With various comparison, no difference in the depression was noted. Chandler (4), Dixon and Atkins (13), Knudson and Ginsburg (26), and Newton (33) have found it desirable to use high pressures in order to express the sap. This procedure seemed unnecessary, since in the first place the tissues at all times were young and hence not highly lignified, in which case it is always shown that the difference is small; in the second place, Knudson and Ginsburg's (26) work shows that when lower pressures are used to express the sap, the results more nearly coincide with the plasmolytic method. Salmon and Fleming (35), working with older wheat, found it necessary to treat the material with toluene or chloroform. The age of the plant also reduces very much the undercooling as discussed by Harvey (21).

Experiments were conducted to determine the effect of freezing this material before extracting the juice, as followed by Gortner and Hoffman (17). Comparisons were made with several sets of plants which were grown and treated in the same way in every respect, except that half of each portion was ground and extracted at once, while the other half was frozen for one and one-half hours before extracting. The material was placed in stoppered bottles. These were placed in quart thermos bottles containing a freezing mixture of salt and ice in the proportion which produces a temperature of 12°C. below zero.

TABLE I.—*Depression of the freezing point of sap from plants which were frozen and from those not frozen, and the difference in each case*

Date	Age of plants	Depression when—		Difference (+) or (−) when frozen	Date	Age of plants	Depression when—		Difference (+) or (−) when frozen
		Frozen	Not frozen				Frozen	Not frozen	
November 17-----	<i>Days</i> 7	0.467	0.475	−0.008	November 6-----	<i>Days</i> 13	0.407	0.390	+0.017
Nov. 19-----	9	.470	.480	−.010	Nov. 13-----	15	.400	.390	+.010
		.360	.395	−.035			.495	.470	+.025
Nov. 5-----	9	.360	.400	−.040			.480	.465	+.015
		.430	.415	+.015					
		.425	.405	+.020	Average difference-----				+.0045

The differences indicate that freezing has no consistent effect in increasing the osmotic pressure of the cell sap, and that the cells are not made more permeable by both freezing and grinding than by grinding only without freezing.

The 2-day-old seedlings formed a paste when ground, but not enough sap could be extracted from this paste to make the test.

Therefore the method as described by McCool and Millar (31) was followed in the determination of the freezing point of the ground tissue. The ground paste was placed in the freezing tube in such a way as to press gently around the bulb of the Beckman thermometer. The only precaution necessary to use in making the determinations in this way is to keep the freezing bath at about 4° C. Experiments on older plants indicate that the depression is slightly greater with the tissues than with the extracted juices, and this confirms the work of McCool and Millar (31). (Data are given in Table II.)

TABLE II.—*Depression of the freezing point of the juice and ground tissue of plants*

Age of plants	Depression in degrees		Increase in depression in ground tissue
	Juice	Ground tissue	
<i>Days</i>			
5	0.75	0.76	0.01
	.715	.765	.05
6	.61	.705	.095
	.62	.710	.090

Since the depression is somewhat greater for ground tissue than for extracted sap, the reading for the 2-day-old plants shows a depression slightly in excess of what we would expect.

In all cases except the 15-day stage with four samples, the results given are from the average of determinations of five sets of plants grown separately, and no more than two determinations were made in any one day.

Comparisons were made of the freezing-point depression of the sap of roots, plumules, and entire plants. The data are submitted in Table III.

TABLE III.—*Comparison of the freezing-point depression of the sap of roots, plumules, and entire plants*

Age of plants	Depression in degrees		Average	Entire plants
	Plumules	Roots		
<i>Days</i>				
10	0.545	0.260	0.4025	0.39
	.545	.250	.3975	.385
15	.600	.255	.4275	.43
	.575	.250	.4125	.43

The plumules have a depression nearly twice as great as that of the roots. If reference is made to Table IV it may be observed that the plumules have more soluble materials and less water than the roots. This would account for the difference in the freezing points. Averaging the depression of the roots and plumules, the depression is practically the same as for the entire plant. This is to be expected, as the green weights of the roots and tops differ by only a little.

TABLE IV.—Carbohydrates, expressed as dextrose, and the nitrogen content of seeds, plumules, roots, and total plants expressed as milligrams, per 100 parts, per cent of dry weight, and per cent on the basis of the original amount in the seed

Parts of plant and age	Weight in grams per 100 parts	Per cent of weight of original seed	Ether extract	Per cent of dry weight	Per cent of either extract of original seed	Per cent of reducing sugars	Per cent of dry weight	Total sugars	Per cent of dry weight	Per cent of total sugar of original seed	Dextrin	Per cent of dry weight of dextrin	Per cent of original seed of dextrin	Starch	Per cent of dry weight	Per cent of starch of original seed	Acid hydrolyzable	Per cent of dry weight	Per cent of acid hydrolyzable of original seed	Nitrogen	Per cent of dry weight	Per cent of weight of nitrogen of original seed
Original seeds:-----	2.085	-----	66.9	2.49	-----	-----	-----	53.86	2.00	-----	43.5	-----	-----	1,781.0	66.40	-----	249.5	9.30	99.0	59.07	2.20	-----
1 day-----	2.708	100.80	63.1	2.52	94.2	-----	-----	44.7	1.50	83.0	82.0	3.03	198.1	1,621.3	59.91	91.10	246.9	9.12	99.0	54.41	2.01	92.20
2 days-----	2.593	96.82	57.6	2.30	86.0	22.76	0.91	137.15	5.29	255.0	111.0	4.28	255.3	1,079.0	41.60	60.59	243.5	9.39	97.6	46.20	1.78	78.22
3 days-----	2.5443	94.80	51.03	1.95	76.4	83.145	3.18	121.39	4.64	225.0	81.29	3.10	186.7	1,343.5	51.30	75.40	241.2	9.22	96.8	48.83	1.92	82.86
6 days-----	2.4761	92.20	45.95	1.85	66.7	234.775	9.48	465.80	18.80	864.2	120.51	4.88	277.2	472.1	19.05	26.50	259.3	10.47	103.8	49.64	2.00	84.02
9 days-----	2.3833	88.80	95.28	4.00	142.4	117.348	4.92	208.975	8.76	387.0	86.53	3.63	198.7	117.5	4.99	6.60	332.7	13.95	133.2	63.31	2.66	107.2
12 days-----	2.0307	75.70	94.71	4.46	141.5	15.59	0.77	37.08	1.82	70.2	19.87	0.78	45.7	20.2	0.99	1.13	300.5	14.77	132.5	54.32	2.67	92.00
15 days-----	2.3554	87.70	122.76	5.25	183.5	5.45	0.17	23.76	1.01	44.1	15.85	0.73	36.4	14.65	0.62	1.82	295.4	12.54	118.5	79.84	2.38	134.90
25 days-----	5.173	192.50	310.20	6.00	494.0	10.06	0.194	12.49	0.24	23.2	13.57	0.262	31.2	64.3	1.24	3.61	484.5	9.36	194.2	177.72	3.44	301.9
Seeds:-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 days-----	2.3317	86.9	46.3	1.99	69.2	76.50	3.28	105.90	4.54	196.0	75.35	3.23	173.1	1,339.5	57.4	75.12	217.2	9.31	87.2	37.56	1.61	63.60
6 days-----	1.8072	67.3	31.2	1.72	46.7	212.70	11.76	430.70	23.81	799.0	105.70	5.85	242.9	468.0	25.9	26.26	210.0	11.6	84.2	27.30	1.51	46.22
9 days-----	0.887	31.2	29.7	3.54	44.4	102.10	12.2	167.00	19.91	310.0	68.06	8.12	155.5	109.0	13.00	6.12	138.3	16.5	55.5	12.75	1.52	21.57
12 days-----	2.266	10.7	16.27	5.88	24.3	8.40	2.93	20.46	7.15	37.2	8.93	3.12	20.5	17.9	6.24	1.01	89.9	31.4	36.0	3.64	1.27	6.16
15 days-----	0.279	10.4	13.65	4.89	20.4	1.25	0.45	3.82	1.37	7.08	4.92	1.78	14.9	10.6	3.80	0.60	88.8	31.8	35.6	2.18	0.78	3.69
25 days-----	0.250	9.3	12.85	5.14	19.2	0	0	0.65	0.26	1.20	2.98	1.18	6.8	5.9	2.36	0.33	8.0	3.2	3.21	0.93	0.37	1.57
Plumule:-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 days-----	0.1096	4.09	2.12	1.93	3.17	3.095	2.82	9.18	8.37	17.00	3.30	3.01	7.6	2.58	2.5	0.14	8.91	8.12	3.57	5.88	5.36	9.95
6 days-----	0.3832	14.3	8.65	2.26	12.92	13.585	3.54	20.42	5.33	37.90	9.98	2.50	22.0	2.86	7.47	0.16	2.86	7.47	1.15	14.80	3.86	25.04
9 days-----	0.8754	32.6	54.80	6.27	81.9	13.55	1.55	26.50	3.03	49.2	11.70	1.34	26.7	7.00	0.80	0.39	95.4	10.90	38.3	37.71	4.31	63.81
12 days-----	1.0536	39.2	68.25	6.48	102.0	0	0	4.09	0.389	7.60	3.03	0.288	7.0	0	0	0	77.8	7.38	31.2	34.80	3.30	58.95
15 days-----	1.4489	54.0	99.90	6.90	148.0	0	0	5.5	0.38	10.2	4.77	0.329	11.0	2.15	0.148	0.12	93.0	6.42	37.2	66.74	4.59	113.00
25 days-----	3.5730	133.2	272.50	7.63	407.5	0	0	0	0	0	0	0	0	21.5	0.602	1.21	182.4	5.10	73.1	143.70	4.02	243.20
Roots:-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3 days-----	0.1030	3.84	2.6	2.62	3.89	3.55	3.45	6.30	6.12	11.68	2.46	2.39	5.6	1.45	1.41	0.08	16.10	15.6	6.46	5.39	5.23	9.13
6 days-----	0.2859	10.6	6.1	2.14	9.12	8.49	2.97	14.76	5.17	27.4	5.23	1.83	12.0	0.61	0.214	0.03	46.48	16.25	18.61	7.54	5.23	12.75
9 days-----	0.4692	17.5	10.7	2.28	16.0	11.73	2.50	15.47	3.30	28.70	6.77	1.44	15.6	1.47	0.398	0.08	98.97	21.05	39.65	12.95	2.76	21.94
12 days-----	0.6905	25.7	10.2	1.48	13.2	7.19	1.04	12.53	1.81	23.40	7.83	1.13	18.0	2.27	0.328	0.11	132.75	19.21	53.21	15.88	2.30	26.89
15 days-----	0.7275	27.1	9.2	1.26	13.7	4.20	0.577	14.44	1.98	26.80	6.10	0.841	14.0	1.90	0.261	0.12	113.55	15.62	45.51	10.92	1.50	18.47
25 days-----	1.3500	50.2	24.8	1.84	37.0	10.05	0.744	11.835	0.876	21.90	10.62	0.786	24.4	36.85	2.73	2.06	204.10	21.80	117.8	33.09	2.45	56.00

The green weight, dry weight, and ash determinations, as shown in Table V and plotted in Figure 1, were made on plants grown as just described. The plants were counted, dried of excess moisture, placed in weighing bottles, then weighed to determine the green weight, and then dried rapidly in a vacuum oven at 80° C. to complete desiccation. The dried plants were then transferred to porcelain crucibles and reduced to constant weight for ash in an electric muffle furnace at dark-red heat. The ash determinations are expressed not only as grams of ash but also per cent ash of dry weight and per cent of weight of the original seed.

TABLE V.—Green weight, dry weight, and weight of ash for 100 parts, expressed in grams, and per cent of ash in dry material, per cent of ash based on ash of original seed, and per cent of dry weight of original seed

Age and plant	Part of green weight	Dry weight	Weight of ash per 100 parts	Per cent ash of dry weight	Per cent ash of original seed	Per cent dry weight of original seed
Original seed (air dry)-----	2. 8990	2. 6228	0. 0534	2. 03	-----	-----
Plumule:						
3-day-----	. 4760	. 0896	. 0059	6. 58	11. 0	3. 42
6-day-----	3. 8260	. 3210	. 0188	5. 84	35. 2	12. 24
9-day-----	6. 4740	. 6401	. 0534	7. 22	100. 0	24. 40
12-day-----	8. 7840	. 8428	. 0910	10. 75	170. 2	32. 13
15-day-----	14. 2085	1. 3961	. 1869	11. 94	350. 0	53. 22
25-day-----	32. 2880	3. 2612	. 3572	10. 95	668. 9	124. 30
Roots:						
3-day-----	. 851	. 0819	. 0063	7. 73	11. 8	3. 12
6-day-----	3. 860	. 2460	. 0229	9. 31	42. 89	9. 37
9-day-----	6. 578	. 4521	. 0465	10. 27	87. 08	17. 23
12-day-----	9. 520	. 4889	. 0628	12. 85	117. 61	18. 64
15-day-----	12. 3648	. 5990	. 0659	10. 99	123. 41	22. 83
25-day-----	16. 787	1. 11850	. 0976	8. 25	182. 77	45. 18
Seed:						
3-day-----	4. 284	2. 3632	. 0471	1. 95	88. 20	91. 62
6-day-----	2. 440	1. 8072	. 0316	1. 74	59. 18	68. 90
9-day-----	3. 066	. 8694	. 0141	1. 63	26. 40	33. 15
12-day-----	1. 995	. 3384	. 0095	2. 79	17. 79	12. 52
15-day-----	2. 014	. 2622	. 0062	2. 36	11. 61	9. 99
25-day-----	1. 308	. 2432	. 0088	3. 60	16. 48	9. 26
Plant:						
3-day-----	5. 611	2. 5745	. 0593	2. 30	111. 05	98. 16
6-day-----	10. 126	2. 3742	. 0733	3. 09	137. 27	90. 52
9-day-----	16. 118	1. 9616	. 1140	5. 81	213. 48	74. 79
12-day-----	20. 299	1. 6701	. 1633	9. 77	305. 60	63. 71
15-day-----	28. 5873	2. 2513	. 2590	11. 50	485. 02	85. 83
25-day-----	50. 383	4. 6894	. 4636	9. 88	868. 15	178. 80

The plants used for the chemical analysis were separated into seeds, plumules, and roots. The material was dried in a vacuum oven at 80° C., which has been found by Link and Tottingham (30) to be a very successful way to treat carbohydrate material for the analysis of sugars. In cases where the sugar content was high, as for example in seeds from the 6 and 9-day-old seedlings, it was found necessary, even under these conditions, to use Petri dishes and have the seeds well separated instead of using the weighing bottles which were used in the other cases, in order to have the drying go on fast enough to prevent discoloration and accompanying chemical changes. It was also necessary to avoid putting large quantities of material in the oven at one time (an amount which would dry enough in six hours to crumble nicely seemed to give the best results). In cases where it was dried more slowly it usually lost the bright, desirable color. This method is preferable to the alcohol method where small quantities

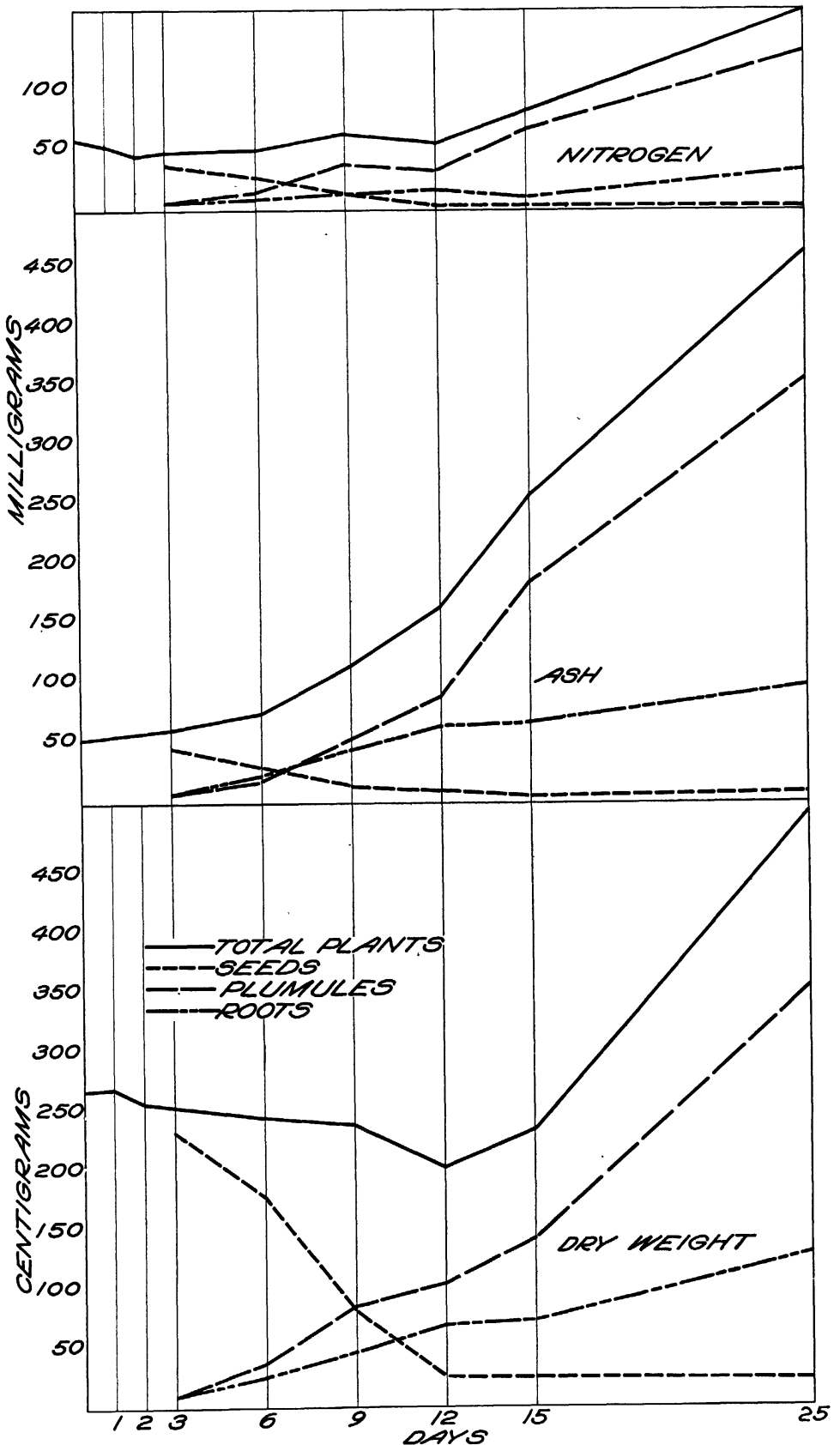


FIG. 1.—Dry weight expressed as centigrams, and ash and nitrogen expressed as milligrams. Solid line—total plants; short dash line—seeds; long dash line—plumules; long and short dash lines—roots

of material are used. Plants for each sample were collected on four or more different days in order to make a composite sample. The samples were ground in a pulverizing mill, often enough so that 90 per cent would pass through a 100-mesh sieve. The sample was then mixed and placed in bottles in a desiccator ready for analysis.

METHOD OF ANALYSIS

Duplicate samples which weighed between 1 and 1.5 gms. were extracted in a small Soxhlet extractor with anhydrous alcohol-free ether. This removed the lipoids and soluble pigments, which were determined by evaporating off the ether from the extract and weighing direct. The residue was placed in a 70° C. oven over night to drive off all the ether. The next day the residue was removed from the extraction shell to a 500 c. c. Erlenmeyer flask, and extracted in a water bath for one-half hour with 150 c. c. of 90 per cent boiling alcohol, to which a little calcium carbonate had been added to neutralize any plant acids which might be present. This was filtered and washed with 90 per cent alcohol. The extract was then reduced in volume under reduced pressure at 50° C., as described by Van Slyke (42, p. 21), to remove the alcohol. It was then diluted with water, clarified with lead acetate, filtered, freed from excess of lead by sodium carbonate, again filtered, and made up to 100 c. c. volume; 50 c. c. were used for the reducing sugar and 50 c. c. for the hydrolyzable sugars.

Reducing sugars were determined by the Munson-Walker method. The gas was regulated to bring the solution to boiling in the regulation time by using an oil manometer on the gas line. In this way it was possible to have the liquid begin boiling within 10 seconds of 4 minutes. The reducing sugars were expressed as dextrose from the Munson-Walker tables given by Leach (27).

Total sugars were determined by hydrolyzing the extracts with 25 per cent HCl; 5 c. c. of HCl with 1.25 sp. gr. was added to 50 c. c. of extract, and boiled for an hour with a reflux condenser. This was neutralized with NaOH. The total sugars were determined by the Defren-O'Sullivan method, and expressed as dextrose.

In order to determine the dextrans, the residue from the sugar extraction was further extracted with 10 per cent alcohol at a temperature of 50° C. for one-half hour in a water bath. This was carried on in the same bath and while the sugars were being reduced in volume at 50° C. The filtered extract was concentrated for the removal of alcohol and clarified by the method just given. After hydrolysis with 25 per cent HCl for 1 hour and neutralizing with NaOH, the dextrin was determined as dextrose by the Defren-O'Sullivan method.

Starch was determined by boiling the residue from the dextrin extraction for 1 minute with 80 c. c. of water, in order to gelatinize the starch. After cooling to 38° C., the starch was digested with fresh saliva as suggested by Link and Tottingham (30), until a negative result for starch was obtained with iodine, then boiled and tested again with iodine; if negative, it was filtered, and the solution was hydrolyzed with 2.5 per cent HCl for 1½ hours, then neutralized with NaOH. The starch was then determined by the Defren-O'Sullivan method and expressed as dextrose. The fresh saliva

seemed more desirable than a commercial product used by Horton (24).

Hemicellulose, or acid hydrolyzable material, was determined by boiling the residue from the starch digestion with 100 c. c. of 2.5 per cent H_2SO_4 for 1 hour, with a reflux condenser. The solution was filtered and the filtrate neutralized with NaOH and clarified as above. The acid-hydrolyzable substance was determined by the Defren-O'Sullivan method and expressed as dextrose.

Total nitrogen was determined by using about 0.7 gm. of material from the composite samples. The Kjeldahl method as modified by Arnold-Gunning, with the further modification to include nitrates by the addition of salicylic acid, was used, and the results expressed as total nitrogen.

DISCUSSION

The dry weight of the total plants after germinating for one day is greater than the dry weight of the original seeds, but during the second and third day a considerable loss takes place. Doyer (14) points out that the chief changes in the first day of germination is the absorption of water, and little heat is given off. The greatest warmth is developed during the third and fourth days. Cribbs found by measuring the CO_2 given off during germination⁴ that the respiration on the second day was about three times as great, and on the third day about five times as great as the first day. This small amount of respiration and loss of weight, with the gain in weight due to the hydrolysis of starch, may account for the slight increase in weight. The seed has the most rapid loss in weight between six and nine days, which is a period of rapid growth, before the plant has developed far enough to make carbohydrates rapidly. It is, however, increasing its ash content. The lowest dry weight is reached at 12 days, after which the anabolism is more rapid than the katabolism. The dry weight of the plumules seems to be always greater than the dry weight of the roots.

The use of storage fat by the seed has been studied extensively on fatty seeds. A comprehensive history is given by Miller (32). Waśniewoski (43) reports an increase in the amount of fat during the germination of wheat seedlings and suggests that some is formed from starch. This is in disagreement with most of the earlier work and with results reported here, which do show, however, that the fats of the seed are used more slowly than the other reserve foods in the seed. Spoehr (37) says "fats are important, especially in the development of seedlings." The other extract decreases slowly in the total plants up to six days, but at nine days a decided increase, particularly in the plumules, takes place. This is probably due to the chlorophyll. By referring to Table IV or Figure 2, it will be noted that at nine days the plants have about established the normal amount of ether extract, since the percentage in the plumules and roots remains about constant from this point on. The roots have a higher percentage of fat in the young stages than in older stages, which suggests that some of the fat may be translocated as fat and is later used by the root.

⁴ CRIBBS, W. J. THE EFFECT OF FREEZING SEEDS ON THE RESPIRATION AND CATALASE ACTIVITY OF SEEDLINGS. 1923. [Unpublished thesis, Univ. Chicago.]

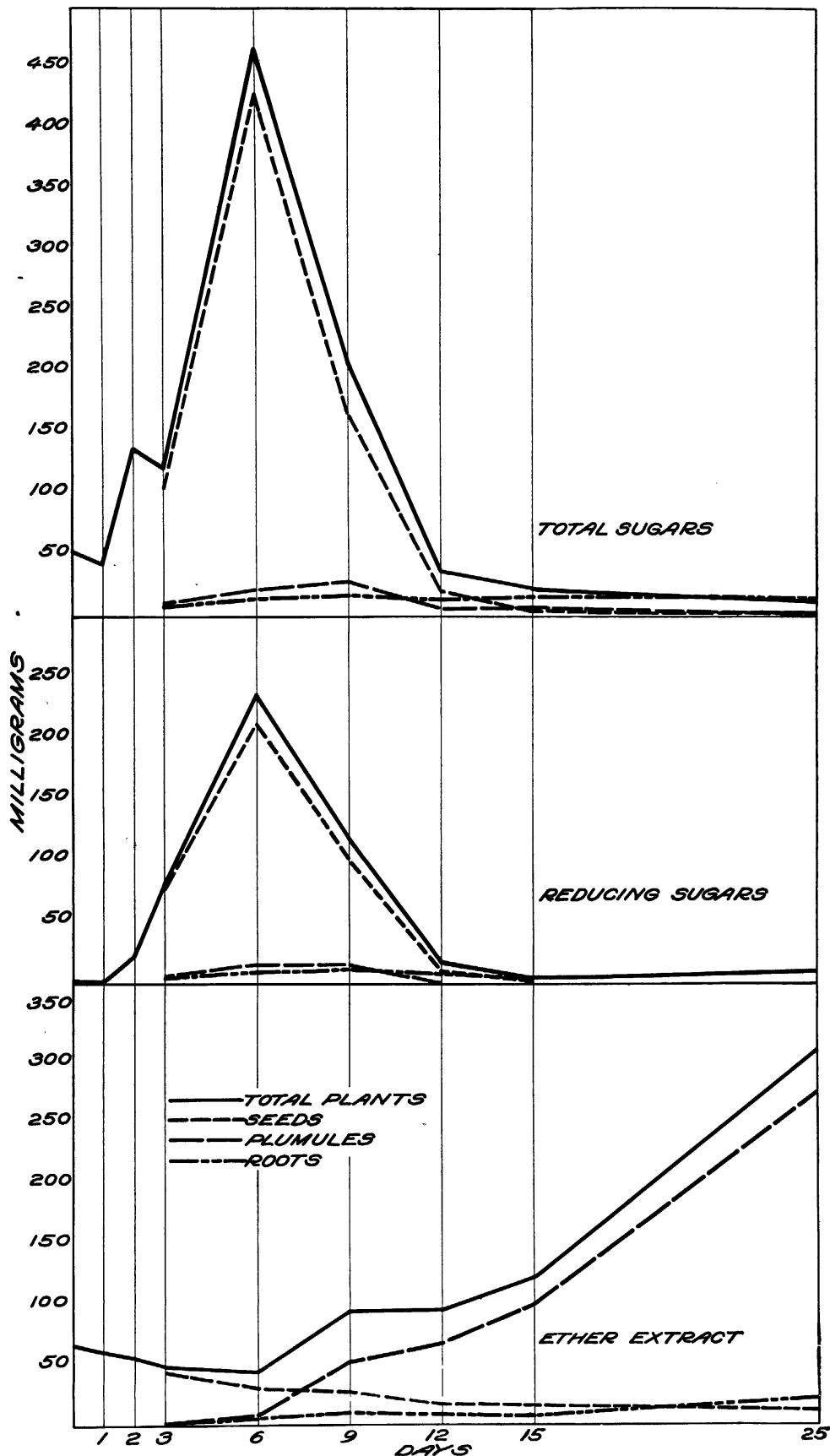


FIG. 2.—Ether extract, reducing sugar, and total sugars, expressed in milligrams. Solid line—total plants; short dash line—seeds long dash line—plumules; long and short dashes—roots

The seed contains no reducing sugar. Choate (5), by micro-chemical means, was able to detect reducing sugars at 18 hours. Under the conditions in which this material was grown, no reducing sugar was found until the second day. At two days there is good reason to believe the sugar to be maltose rather than a hexose sugar. In fact at no time is there evidence of more than a very small amount of hexose sugar. Le Clerc and Breazeale (28) found the amount of reducing sugars in the axes increased up to about the ninth day, when 100 axes (plumules and roots) contained 262.3 milligrams of reducing sugar. The greatest amount of reducing sugar shown by this work is also at the ninth day, but the amount is only 25.3 milligrams in 100 axes. The reducing sugar in the seed in the two cases parallel each other remarkably well, both in the maximum amount and in the increase and decrease. The source of the difference in these results must, therefore, lie first in the rate at which the starch is hydrolyzed in the seed, being more rapid in the case of plants grown in water cultures than in the case of plants grown in soil. The excess sugar formed in water cultures must then be translocated to the axes more rapidly than it is required by those organs, while, when grown in soil the translocation is proportional to the rate it is used. Le Clerc and Breazeale (28) have shown nearly equal percentages of sugars in the axes and in the seeds, while these results show 12 per cent of sugar in the seeds and less than 2 per cent in the axes, in 9-day-old plants. Green (18) found invert sugar to increase during 13 days germination of castor-oil beans. Miller (32) reached the same conclusion with *Helianthus annuus* seeds. Newton (33) reports larger quantities of reducing sugars in leaves of hardened winter wheat.

The data here presented as total sugar show no evidence of sucrose formation taking place in the seed. The plumules appear to have sucrose in addition to sugars of translocation. Brown and Morris (3), working with barley, were unable to find that maltose passes from the endosperm to the embryo, but assumed that it changed to simple sugar during translocation. When a larger amount of sugar is found after hydrolysis than before, it is assumed that sucrose is present. Le Clerc and Breazeale (28) did not determine the latter point, but they agree that no sucrose is formed in the seed. Colin and Belval (7) find sucrose to increase regularly in the stem. Translocation has taken place more rapidly into the roots than the sugar is used. Newton (33) finds as much as 16 per cent of the dry weight of winter-hardened winter wheat leaves to be sucrose. Green (18), in castor bean, finds first a decrease and then an increase of sucrose up to 13 days. Choate (5) finds no maltose in the seed.

The dextrans follow very closely between the starches and sugars, of which they are intermediate products. Where the starch shows a rapid loss the dextrans increase, and they drop off at about the same rate as the total sugars. At no time is there enough dextrin in the axes to indicate that translocation of dextrans takes place. Lindet (29), working with germinating barley, reports no dextrin, but suggests that the starch is changed to sucrose directly.

Starch is the principal storage polysaccharide of wheat, although a certain amount of tritacin is reported by Thatcher (41). Spoehr (37) points out that it is not the total amount of carbohydrates, but the

degree of chemical inversion that determines the supply of material available for respiration and growth. Waśniewski (43) reports a constant relation between the starch decomposed and that consumed in respiration, regardless of the temperature, light, and changes in nitrogenous and inorganic substances. Doyer (14) says "a great loss of energy takes place the first seven days, mostly the third and fourth days." In the work here presented, the graph of Figure 3, showing the starch content, drops rapidly the first seven days, but the decline is extreme from the third to the sixth days. The sugars are highest at six days. The proportional increase in the dry weight of the axes was greatest at this period. All these facts substantiate the conclusions of the above investigators, showing that the greatest starch hydrolysis and greatest sugar content accompany greatest growth.

Small amounts of starch are found in the axes at all stages here examined, but with a slight increase at the 25-day stage. Newton (33) found no starch in the leaves of winter wheat when hardened, Wimmer (44) found starch in all parts of the wheat plant which were about 6 weeks old—about 2 per cent in the leaves, 6 per cent in the stems, 2 per cent in the roots, and an average for the whole plant of 3.5 per cent. Choate (5) found no starch in the plumule or the root; however, her seedlings were kept in the dark. In considering the evidence just given, it would appear that if the seedling has good growing conditions it will store starch in all the tissues, but if conditions are not favorable no starch will be found.

The acid hydrolyzable or hemicellulose content of wheat seedlings is made up largely, according to the classification given by Sherman (36) of xylem, found in the seeds and the tissues of plumules and roots. The percentage decreases slightly in the first three days, due to the action of cytase on the cell walls of the endosperm; after this the percentage based on dry weight increases, denoting that the hemicellulose is largely in the seed coverings which are not used. The percentage of hemicellulose remains about constant in plumules and in roots, but is twice as great in the roots as in the plumules. The total amount of acid-hydrolyzable substances expressed as dextrose increases from 3 per cent.

The nitrogen is not converted into protein by multiplying by a factor, since it is felt that, while it would be desirable to express the nitrogen of the seed as protein, which is described by Osborne (34), it would be incorrect to use the same factor for the nitrogen of the roots, which are high in nitrogen of other forms. Deleano (12) has shown, with leaves of *Vitis vinifera*, that as long as carbohydrates are present the protein is not used in respiration. Davidson and Le Clerc (8) find that the amount of nitrogen in the plant depends somewhat on the soil; when nitrogen is low in the soil the plants are usually lower in nitrogen. Gericke (16) has found a similar relation.

Table IV and Figures 1 and 3 show that the seed loses the nitrogen more slowly than the starch. The protein remains nearly constant in percentage of dry weight until the ninth day, which is the age at which the carbohydrates become deficient. Choate (5) found that the proteins of the endosperm are the first to be used, while the protein of the aleurone layer remains until very late in the germination stages. A large amount of nitrogen is translocated, as shown by the

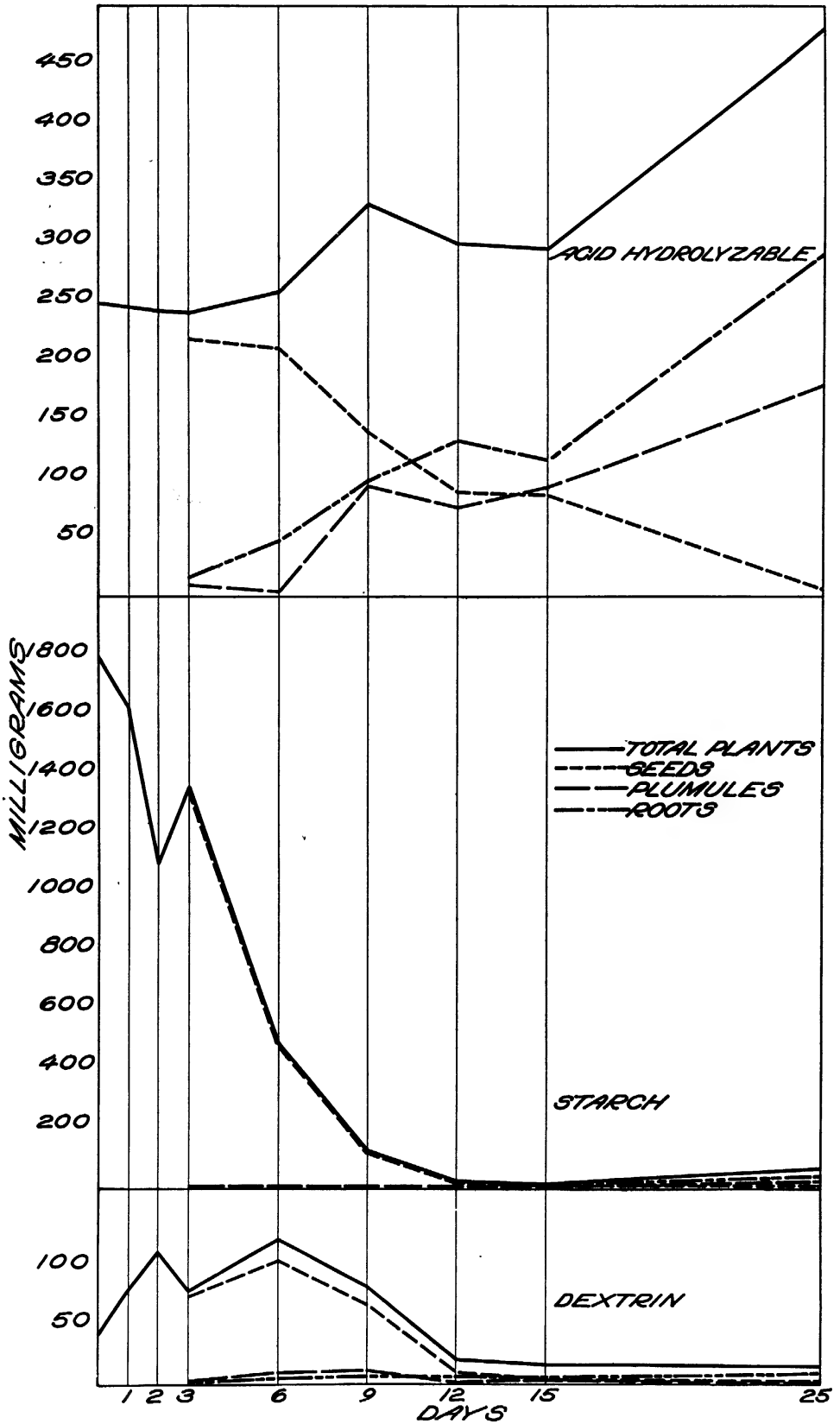


FIG. 3.—Dextrin, starch, and acid-hydrolyzable substances, expressed as milligrams. Solid line—total plants; short dash line—seeds; long dash line—plumules; long and short dashes—roots

high percentage of that element in 3-day roots and plumules with proportional loss in the seed. At 6 days the total plant has almost the same percentage of nitrogen as the original seeds and after that the percentage is always higher.

Haigh (19) concludes that wheat takes up nitrogen very rapidly in the seedling stage. Hoagland (23) has obtained results indicating that barley acts in the same way with respect to nitrogen during the early growing stages.

The ash of wheat seedlings changes more rapidly, and the maximum increase is greater, than the nitrogen or the carbohydrate content. The ash of the plumules and roots expressed as per cent increase over that in the seed is conspicuous, due to its rate of increase. A 3-day-old root with a dry weight of less than a milligram, which is 3 per cent of the original seed, is nearly 12 per cent ash. Thus the minerals are deposited in the roots more than fourtimes as rapidly as the other substances. Plumules of the same age are a little heavier and contain 11 per cent ash. The close agreement in the amount of ash in the roots and plumules indicates that the minerals which are absorbed from the soil by the roots are translocated at about the rate that they are taken up. The total ash of 3-day-old plants was 11 per cent greater than that of the original seeds. The leaching from the seeds to the soil, if such a loss take place in soil as is described by Le Clerc and Breazeale (28) in water cultures, must be recovered by the plant at three days, besides the total increase which is found, hence the total intake of minerals is exceedingly large for this early development. The ash is not used from the seed as rapidly as the organic material, and the per cent of ash in the seeds falls slightly for nine days, then increases. Nine-day-old plumules have as much ash, and the 9-day root nearly as much ash, as the original seed, while the dry weight is less than one-fourth as great. The percentage of ash in the plumules continues to increase up to 15 days, when it is nearly 12 per cent, while in the roots the maximum percentage is attained at 9 days. Haigh's (19) work indicates a rapid intake of nutrients in the young wheat seedling. While Wimmer (44) found in the first crop of plants—that is, those a little larger than the ones used here—8 per cent ash; and when ripe the content was 2.33 per cent. The apparent small increase in the ash of 25-day-old seeds may be due to the fact that at this time much infection of seed has taken place and the lower organisms may be adding ash.

The rapid intake of nitrogen and the mineral elements by the young wheat seedling indicates that the plant is able to utilize the largest amount of these elements during the first few weeks of growth, and that the mineral supply is the limiting factor for the development in these young stages. Minerals, Figure 1, are the only substances which actually increased as early as the third day. It may be mentioned that in common agricultural practice fertilizers with a relatively high amount of nitrogen are applied to soils low in fertility in order to give the wheat a more rapid early growth. Davidson and LeClerc (8) found that early applications of nitrogen increased the yield. Osmotic pressures are given according to the work of Harris and Gortner (20).

TABLE VI.—Concentration of the expressed cell sap

Age of plants (days)	Length in centimeters		Dry weight in grams	Freezing point of expressed juice, depression in °C.	Osmotic pressure in atmospheres
	Tops	Roots			
2	0.15	0.7	0.0257	0.8910	10.73
3	.50	1.5	.0247	.8135	9.795
4	1.40	5.2	.0244	.676	8.021
5	3.00	6.3	.0225	.646	7.76
6	4.7	8.0	.0196	.5877	7.08
7	6.5	10.6	.0199	.552	6.65
8	8.5	14.4	.0178	.4995	6.02
9	9.4	15.6	.01939	.4555	5.367
10	11.5	16.3	.01905	.4205	5.068
11	11.7	16.3	.0193	.4170	5.026
12	12.2	18.5	.0201	.4245	5.116
13	13.2	20.0	.0195	.4030	4.857
14	13.6	19.5	.0210	.4275	5.272
15	16.4	20.9	.0209	.445	5.362
25	19.5	27.8	.0396	.497	5.99

If one follows in Table VI or Figure 4 the depression of the freezing point of the cell sap of wheat seedlings, it will be noticed that there is a

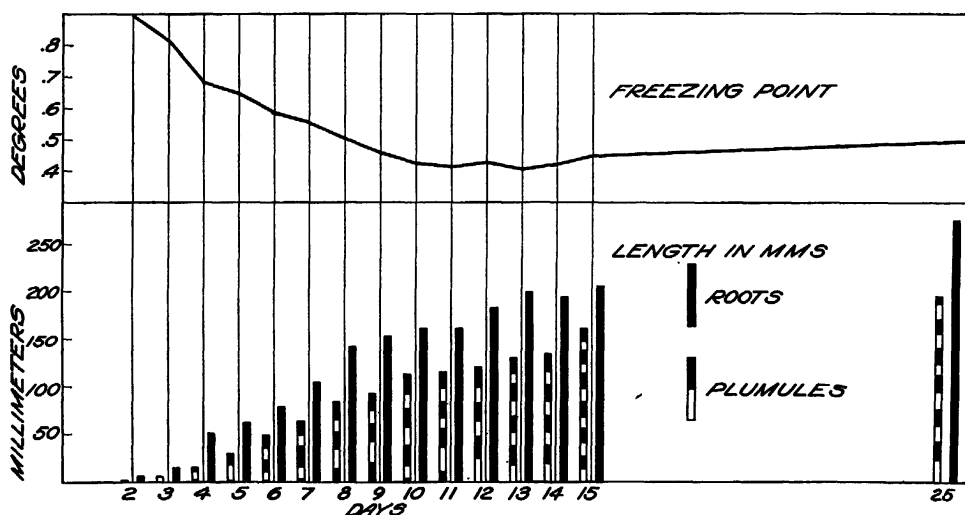


FIG. 4.—Length of roots and plumules, in millimeters; and freezing-point depression of the cell sap, in degrees

gradual decrease from the 2-day-old plants to about the 12-day-old plant stage, after which the osmotic pressure becomes greater. The depression of the freezing point is obviously due to the factors already discussed—namely, the amount of water and soluble substances. The amount of moisture becomes greater after the 2-day stage, which tends to decrease the osmotic pressure. The rôles played by the sugar and ash elements reach their maximum values at different stages. In the 3-day stage the ash is very high, being nearly 2 per cent of the moisture content; the sugar is lower at the 3-day stage than at the 6-day stage. The percentage of ash, based on moisture content, drops from the 2-day-old stage to the 9-day-old stage, from which point there is a continual increase. The sugars gradually but continuously decline in percentage. Bakke's (1) work indicates by the rate of transpiration that the plant contains more water in the morn-

ing. Yuncker (46) found the maximum photosynthesis took place in the corn plant about noon. These two factors would tend to increase the depression of the freezing point of stages effected by transpiration and photosynthesis if the plants were collected during the middle of the day and bring about less difference in the 2-day-old stage and later stages. These conditions could not be controlled from day to day; therefore it was thought advisable to avoid that period. The morning collections of material obviously give more accurate results on the plant's composition.

SUMMARY

During the early stages of the germination of the wheat seedling, the ether extract disappears from the seed more slowly than the carbohydrates or nitrogen, and accumulates in the plumules and roots more slowly than any other food material investigated.

The small amount of sugars normally found in the wheat kernel increases rapidly in the seed during the first six days of germination when nearly three-fourths of the starch has been used. After six days, the amount of sugar decreases. The sugars are translocated to the plumules and roots more rapidly than they can be used, while there is a supply of starch in the seed, but after nine days there remains approximately 1 per cent of the original starch; the sugars in the axes decrease sharply. These plants are dependent upon their photosynthetic powers for carbohydrates after 12 days. Under the conditions of this experiment, the embryo seemed to depend on the endosperm for about six days for its carbohydrate supply. During the period between 6 and 12 days the plant seems to become independent.

The hemicelluloses appear to be of little importance to the developing plant. The plumules and radicles show no marked difference in the percentage of acid hydrolyzable materials during the germination period.

Nitrogen is translocated rapidly in the three-day seedling, but after that the percentage remains constant in the axes. The nitrogen content of the seed decreases in proportion to the weight the first nine days; after that a more rapid loss occurs.

Wheat seedlings require proportionally larger quantities of minerals than any other food material. The maximum ash content of plumules and radicles is reached in 12 to 15 days. The roots appear to take in minerals more rapidly than they are translocated in the early stages of germination.

The concentration of the cell sap decreases from 2 days to 12 days, after which an increase takes place. This follows closely the changes in water, sugar, and ash contents of the plant. No elaborate method of extracting the sap was found necessary.

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POSSIBILITIES AND LIMITATIONS OF CHLOROPICRIN AS A FUMIGANT FOR CEREAL PRODUCTS¹

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INTRODUCTION

There has been a demand for a substitute for carbon disulphide for fumigating cereals and other stored food products to kill the insects which infest them. Carbon disulphide had many satisfactory properties, but its inflammability led insurance underwriters to adopt a ruling which renders an insurance policy void if carbon disulphide is used or stored on the premises of an insured building, and, likewise, practically all railroads have ruled against the use of it in their freight cars.

The great amount of research work which was done on poisonous gases during the war has been drawn upon by workers in nearly every country, but it has not been easy to find a material sufficiently toxic to insects which would not at the same time be injurious to the materials to be fumigated.

This paper is devoted to the results of a study of the toxicity of chloropicrin to certain insects which infest cereals, and to the effect of the chloropicrin on the cereals and their products.

The chloropicrin used in the experimental work was obtained from the Edgewood Arsenal. It had a specific gravity of 1.6595, compared with water at 20° C. Bertrand (3)³ states that chloropicrin prepared by the action of calcium chloride on picric acid has the following properties: Density at 16° C., 1.666; boiling point at 766 mm. pressure, 112° C.; vapor tension at 15° C., 30.2 mm.; non-inflammable; 1.65 gms. soluble in a liter of water at 18° C.

TOXICITY TO INSECTS

SUMMARY OF LITERATURE

The toxicity of chloropicrin to insects was noticed by Moore in 1917 (15) while he was making a comparison of the volatility of organic compounds with their toxicity to insects. His table showed that, on the basis of the amount required to kill flies in 400 minutes, a gram molecule of chloropicrin was about 283 times as toxic as a gram molecule of carbon disulphide. He suggested that its toxicity was very likely due to its action as an enzyme poison.

A second paper by Moore in 1918 (16) gave the results of some experiments to determine the effects of chloropicrin on some of the insects which infest stored food products, on some of the products themselves, on the germination of seeds, and also on certain fabrics which are used for clothing and other purposes. The experiments were performed, for the most part, in wooden boxes, and the report shows

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³ Reference is made by number (italics) to "Literature cited," p. 759.

only the amount of material required to kill in 24 hours. Moore concluded that one-half pound of chloropicrin per 1,000 cubic feet was sufficient to kill most insects, and that 1 pound would give satisfactory results with all those tried.

Since 1918 many papers have been published on this subject. In general, they are devoted to work on the various phases of toxicity on one hand and to use of the fumigant under practical conditions on the other. Notable among the papers of the first group are those by Bertrand and his associates in 1919, 1920, and 1921 (3-9). Those writers showed that as regards the toxicity of chloropicrin to insects, time and concentration bear an inverse ratio to each other. Bertrand also called attention to the fact that there was greater variation due to temperature than would be expected on the principle of Van t'Hoff and Arrhénus. He believed this to be due to the fact that much of the chloropicrin was retained in the tissues and continued to act after the insects had been removed from the gas, thus rendering the observation inaccurate so far as the time factor was concerned. It is interesting to note that when his results for toxicity with a concentration of 20 grams to the cubic meter are plotted against time and temperature, the results fall along a straight line continuing down to 0° C. This is noteworthy when one considers that nearly all other fumigants have their toxicity so reduced at 12° or 15° C. that they are of little practical value at these temperatures.

The results of other investigators are in accord with these of Bertrand and his associates, with regard to the toxicity of chloropicrin, and there is agreement that the effect of the chloropicrin is probably due to action on enzymes. Remy (18) described paralysis of certain individuals of *Argas reflexus* which survived fumigation; and Spencer (19) believed that the action was cumulative, resulting in the death of nearly all of the insects which at first gave evidence of having survived the fumigation.

Regarding the practical use of chloropicrin we have the results of Burkhardt (10), Feytaud (13), Remy (18), Spencer (19), Wille (20, 21), Delassus (12), and others. Here we do not have the general agreement as to its promise that we have with regard to the laboratory experiments on its toxicity. Delassus (12) and others advocate greater use of chloropicrin, and express the belief that the technique of using it needs improving. Burkhardt (10) reports poor results under conditions similar to those which gave good results with carbon disulphide and concludes that chloropicrin might be suitable for use in small receptacles which could be tightly sealed, but that it is not suitable for use on a large scale.

TOXICITY EXPERIMENTS

In the present writers' work, the first series of experiments was performed to determine the toxicity of chloropicrin under laboratory conditions. The chloropicrin was measured out in a small calibrated pipette and placed in a small stoppered vial until the insects had been introduced into glass jars of 8-liter capacity. The insects were placed in small bolting-cloth bags and suspended in the jars by means of threads, the ends of which hung over the edge of the mouth of the jars. The edges of the jars were greased with vaseline in order that there might be no leakage about the threads when the glass cover was in place.

Ten insects were placed in each sack and the sacks were suspended in the jars. The stopper was then removed from the small vial containing the dose which had previously been measured out, and the chloropicrin was carefully sprinkled over the bottom of the jar. The vial was then dropped to the bottom of the jar and the glass cover put in place.

The insects were watched carefully and the first sack of insects was withdrawn as soon as all movement had ceased. The remaining sacks were withdrawn in accordance with a schedule which was arranged on the basis of the time required for the movement to stop. As soon as a sack was withdrawn it was placed in the open air and left for a period of 48 hours, at the end of which time the per cent of dead insects in each sack was noted. As a rule, at least some of the insects revived in the sack which was first removed from the jar. Each successive sack usually had fewer survivors, until finally there were none. The time of exposure of the first sack in which there were no survivors was taken as the time required for 100 per cent kill for the concentration used in the experiment. In general, the decrease in the number of survivors was much more regular in the successive sacks when a high concentration was used than with a low concentration. The controls were left in sacks in the laboratory and were counted with the beetles from the experiments.

The variation found when using some of the lower concentrations was often very confusing, and this may help to explain some of the discrepancies met with in the practical application of chloropicrin. In one experiment there was a difference of more than 15 per cent between two different sacks in the time required to kill 100 per cent. Since no such irregularities were observed in the controls, these differences may have been due to certain sacks becoming wet with the chloropicrin when the dose was introduced, or to some of the insects closing their spiracles for a time when first put into the jar.

The granary weevil, *Calendra granaria* L., was used in the experiments reported in this paper. Bertrand (3) used the rice weevil *Calendra oryza* L., but this species was not available during the present work, so the granary weevil, being very closely related and therefore assumed to be comparable in susceptibility to chloropicrin, was chosen. The results are plotted in Figure 1. Time is recorded in minutes, and concentration in grams per cubic meter. The crosses represent the records of Bertrand (3), and the dots the results of the present work. Bertrand (5) states that the temperature varied between 20° and 27° C. during his experiments, while that in this present work varied between 22° and 24° C. The results are in general accord when it is considered that Bertrand had a variation of 7° in the temperature under which he worked. The inverse relationship between time and concentration seems to hold from the lowest to the highest concentration.

Figure 2 shows the relationship of the time and temperature factors at a concentration of 20 grams per cubic meter. Again the dots represent the results of the present experiments, and the crosses those of Bertrand (6). It is clear that time, temperature, and concentration are important factors. A low value for any one may be compensated for by giving a correspondingly high value to one or more of the others. No relationship was found to exist between relative humidity and toxicity.

The susceptibility of various species of insects to chloropicrin is different. The results of Moore (16), Bertrand (5), and others agree with those of this present work in showing that the confused flour beetle, *Tribolium confusum* (Duval) is more resistant than the weevils worked with, from 30 to 50 per cent greater dosage being required to kill it.

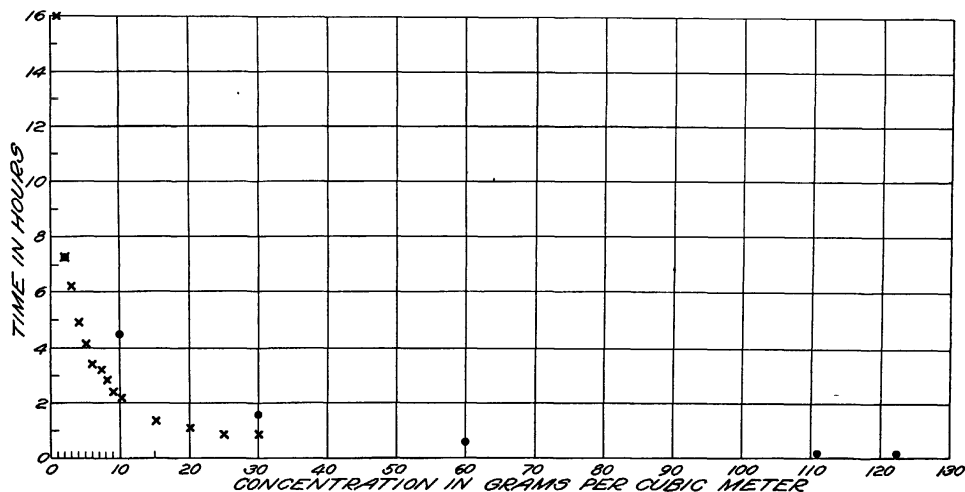


FIG. 1.—Time and temperature curves for toxicity of chloropicrin to *Calendra granaria* L. (used by these writers in this work) and *Calendra oryza* L. (used by Bertrand (3)). Dots represent results of the present work, and crosses those of Bertrand

EXPERIMENTS IN PRACTICAL APPLICATION

The experiments with the practical application of the chloropicrin have had to do very largely with the technique of application. In general, it has been found that the material gives a quicker and more

uniform action when it is atomized. A pressure sprayer which would throw a fine mist has been considered the most satisfactory means of application.

No satisfactory method of measuring the rate of penetration of chloropicrin has been found. However, chloropicrin has been observed to have unusual powers of penetration. The confused flour beetle has been killed in the center of a 98 pound sack of flour in less than 24 hours, with a concentration of about 32

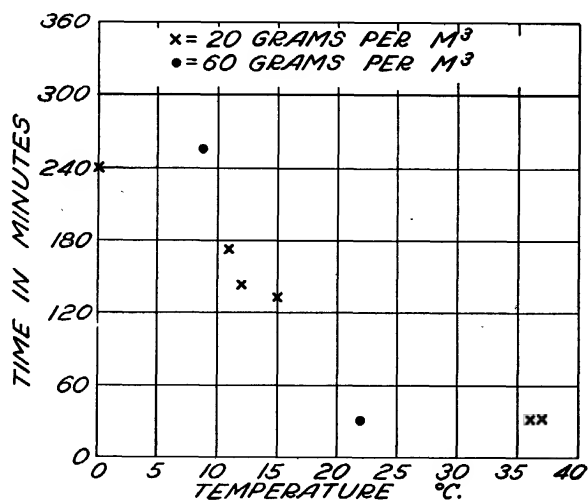


FIG. 2.—Time and concentration curves for toxicity of chloropicrin to *Calendra oryza* L. and *Calendra granaria* L.

gms. per cubic meter in a wooden box. This same property, however, makes it possible for the chloropicrin to escape through the walls of the fumigation chamber more readily than other fumigants do.

After experiments in 1920, Burkhardt (10) concluded that chloropicrin was unsatisfactory for fumigation on a large scale, but he used only 10 gms. per cubic meter, a lower concentration than that

used by Moore and in this present work. Furthermore, in one case he merely poured the chloropicrin over sacks of grain, after which the pile was covered with a tarpaulin. It is not surprising that the small amount of chloropicrin used was soon reduced by diffusion to the point where it failed to kill.

In the course of these experiments, a room 80 by 18 feet was fumigated to kill the Indian-meal moth, *Plodia interpunctella* Huebn. The walls of the room were cracked, and the larvae had gone into the cracks to pupate. All of the walls and ceiling and floor were sprayed with chloropicrin at the rate of about 32 gms. per cubic meter for the volume of the room. The adult moths dropped to the floor as soon as they were touched by the spray, and the larvae and pupae were subjected to an atmosphere saturated with chloropicrin at the start. All the insects were killed. The room was aired by means of an electric fan placed in the only window.

In another case, the empty grain bin of an elevator which was infested with the granary weevil was treated in the same way. Here again the insects were in cracks in the wall. This method subjected them all to an atmosphere which was practically saturated with chloropicrin at the start, which resulted in their death in a very short time. At the same time the concentration was high enough in all parts of the bin to kill any insects which failed to come into contact with the liquid.

When infested grain was placed on the floor of an open box and sprayed with chloropicrin, the insects actually hit by the spray were killed while others 2 feet above the floor survived.

From these experiments it seems evident that, by virtue of its penetration property, chloropicrin is able at the same time to penetrate materials which contain insects, and to escape through the walls of the fumigation chamber so rapidly that its concentration may soon fall below the lethal point. When the material is applied as a spray, the surface of the chloropicrin is so increased that it volatilizes very quickly, thus building up a high concentration in the atmosphere before it begins to escape. Also, the spray can be directed to the infested areas, thus giving the insects a saturated atmosphere at the start.

EFFECTS OF CHLOROPICRIN ON FUMIGATED CEREALS

In the use of any material as a fumigant for cereals, not only are its toxic effects on the infesting organism and its freedom from fire hazard of importance, but worthy of equal consideration are its effects, physical and chemical, on the material fumigated. When wheat or flour which is to be used in making bread is the particular cereal fumigated, these effects may be differentiated into at least two phases. The first of these concerns the effects of the fumigant on the colloidal nature of the proteins of the flour as indicated by a physico-chemical study of such proteins. The second has to do with the effects on yeast activity, in particular on the rate of production of carbon-dioxide gas by the yeast organism. In this paper the use of chloropicrin as a fumigant will be considered from the several angles indicated.

As a fumigant for weevil-infested wheat and flour, chloropicrin has been used to some extent, but previous observations as to its effects on these materials have been of general nature and hence it seemed desirable to undertake a more critical study. Moore (16) observed that chloropicrin had some effect on bread prepared from chloropicrin-fumigated flours. His data tended to show that yeast activity was retarded in the fermenting dough, and that the gluten was affected to some extent, for he noted that the texture of the bread baked from treated flours was slightly inferior to that made from untreated flour.

Ryo Yamamoto (22) stated that when chloropicrin was sprayed into a storehouse used for rice and wheat, noxious insects were killed without bad effects on the cereals. That the fumigant had considerable penetrating ability is indicated by the fact that it killed pupae in cocoons. Piutti (17) stated that chloropicrin gave excellent results in killing insects with no injurious effects on wheat, flour, or bread. Bertrand and Rosenblatt (4, 9) found that very small amounts of chloropicrin retarded and even inhibited enzyme action in general and fermentation in particular. It seems to the writers that this retardation of zymase activity is of importance in bread making.

EFFECT OF YEAST ACTIVITY

Bertrand and Rosenblatt (9) presented data which showed that 6 milligrams of chloropicrin completely inactivated zymase in a liter of the stock yeast suspension which they were using. These workers measured the activity of the enzyme in terms of sucrose which disappeared from the medium.

The manifest function of yeast in bread making is the production of carbon-dioxide gas and aeration of the dough. In this work, the carbon dioxide produced in unit time rather than the percentage of sucrose which was fermented was taken as the measure of yeast activity. A stock solution was prepared containing the following materials: 1,000 c. c. water, 30 gms. yeast, 100 gms. sucrose, and a trace of dibasic ammonium phosphate. To 50 c. c. aliquots of this solution were added varying quantities of aqueous saturated chloropicrin solution, as shown in Table I and II. This aqueous chloropicrin solution was in contact with liquid chloropicrin at 18° C., and as chloropicrin is soluble in water to the extent of 1.65 gms. per liter at this temperature, each cubic centimeter of aqueous chloropicrin solution contains 1.65 mgms. of chloropicrin. Portions of the suspensions containing the several quantities of chloropicrin were placed in fermentation tubes, and the quantity of carbon dioxide read at hourly intervals. The relative quantities of carbon dioxide are given in millimeters of tube length, the fermentation tubes not being graduated. The fermentation was carried on at 28° C.

It was thought desirable in practical fumigation to use a solution of equal quantities of carbon tetrachloride and chloropicrin. Such a solution was prepared, and to it was added distilled water. Quantities of this aqueous carbon tetrachloride-chloropicrin solution were added to 50 c. c. aliquots of the stock yeast solution. The results are given in Table II.

TABLE I.—*Effect of chloropicrin on yeast activity, as indicated by the quantity of carbon dioxide evolved*

Aqueous chloro- picrin solution per 50 c. c. yeast suspension	Chlo- ropic- rin	Height of column of CO ₂ , in millimeters					Aqueous chloro- picrin solution per 50 c. c. yeast suspension	Chlo- ropic- rin	Height of column of CO ₂ , in millimeters				
		1 hour	2 hours	3 hours	4 hours	5 hours			1 hour	2 hours	3 hours	4 hours	5 hours
<i>C. c.</i>	<i>Mgms.</i>						<i>C. c.</i>	<i>Mgms.</i>					
Control.....		93	225	338	425	-----	1.50.....	2.475	30	66	93	133	----
0.1.....	0.165	65	160	260	367	-----	2.00.....	3.300	18	41	58	72	----
.25.....	.412	62	150	232	327	-----	3.00.....	4.950	5	10	15	19	40
.50.....	.825	54	140	230	327	-----	5.00.....	8.250	0	0	0	-----	0
.75.....	1.237	51	140	210	279	-----	10.00.....	16.500	0	0	0	-----	0
1.00.....	1.650	37	80	152	203	-----							

TABLE II.—*Effect of aqueous carbon tetrachloride-chloropicrin solution on yeast activity, as indicated by the quantity of carbon dioxide evolved*

Aqueous carbon tetrachloride- chloropicrin solution per 50 c. c. yeast sus- pension	Height of column of CO ₂ , in millimeters					Aqueous carbon tetrachloride- chloropicrin solution per 50 c. c. yeast sus- pension	Height of column of CO ₂ , in millimeters				
	1 hour	2 hours	3 hours	4 hours	5 hours		1 hour	2 hours	3 hours	4 hours	5 hours
<i>C. c.</i>						<i>C. c.</i>					
Control.....	95	240	377	480	-----	4.0.....	28	62	87	100	-----
0.2.....	70	185	315	402	-----	5.0.....	5	30	58	70	83
.5.....	55	155	266	385	-----	7.0.....	0	4	10	13	25
1.0.....	50	145	226	296	-----	9.0.....	0	0	0	0	0
2.0.....	42	124	232	355	-----	12.0.....	0	0	0	0	0
3.0.....	35	100	160	218	-----	15.0.....	0	0	0	0	0

Tables I and II show the retarding effect of chloropicrin on yeast activity in all the concentrations used. An aqueous solution of carbon tetrachloride had no effect in decreasing the rate of fermentation, hence the retardation and complete inactivation of the enzyme in the case of aqueous carbon tetrachloride-chloropicrin solution was apparently due to the chloropicrin alone. The lesser toxicity of these solutions may be ascribed to a smaller quantity of chloropicrin in the water layer when used with carbon tetrachloride as compared with a saturated solution of chloropicrin alone. In the cases in which aqueous chloropicrin solutions were used, 5 c. c. (8.25 mgms. of chloropicrin) was sufficient to completely stop yeast activity in 50 c. c. of a 3 per cent yeast suspension. When aqueous carbon tetrachloride solutions were used it required 9 c. c. to effect this result.

EFFECT OF CHLOROPICRIN ON GLUTEN

As the literature contained no information concerning the effects of chloropicrin on the proteins of flour, a series of experiments was carried out to determine some of these effects. (Results in Table III.) For purposes of comparison, glutens were prepared from an untreated flour following the methods of the A. O. A. C. (1, p. 169). These glutens were normal in every respect.

TABLE III.—*Effect of chloropicrin on glutens washed from treated flours*

No	Treatment	Wet gluten per 100 gms. flour	Dry gluten per 100 gms. flour	Water held by gluten from 100 gms. flour
		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
1	Control, untreated.....	36. 3976	12. 5760	23. 8216
2	do.....	36. 3424	12. 5160	23. 8264
3	Treated with chloropicrin vapor and aerated.....	33. 5232	12. 3352	21. 1880
4	Treated with chloropicrin vapor and not aerated.....	31. 5992	11. 9352	19. 6640
5	do.....	34. 8200	13. 6784	21. 1416
6	Treated with aqueous chloropicrin solution.....	30. 4440	11. 6880	18. 7560

For the study of the effects of the chloropicrin on the gluten, portions of this same flour were treated in several different ways and glutens were washed from these treated flours. The gluten represented by No. 3 in Table III was prepared from a flour treated in the following manner: 700 gms. of the control flour was placed in a 3,000 c. c. Erlenmeyer flask, and chloropicrin soaked up in filter paper was placed above the flour, care being taken that no liquid chloropicrin should come in contact with the flour. The flour was allowed to remain in the flask for 3 weeks. At the end of this time the flour was removed from the flask, spread out in a thin layer, and breezed with an electric fan for 9 hours. The flour was exposed to the air for 24 hours longer, and then portions of it were used for the experiment.

A comparative study of the gluten washed from this treated flour and that washed from the control flour indicated that the quantity of dry gluten obtained from the two flours was approximately the same—12.5 and 12.3 gms. of dry gluten from 100 gms. each of the control flour and the treated flour, respectively. There was, however, a significant difference in the quantity of moist gluten obtained from these two flours, the moist gluten prepared from the control flour having the greater weight. This meant that the chloropicrin treatment affected the water-holding capacity of the flour. Hence, comparing these same two flours again, the gluten prepared from 100 gms. of the control flour was capable of holding 23.8 gms. of water, while that prepared from the same quantity of treated flour held but 21.2 gms.

Nos. 4, 5, and 6 in Table III show the same effect of the chloropicrin in varying degrees. Nos. 4 and 5 represent glutens washed from nonaerated chloropicrin-treated flours, while No. 6 represents the gluten washed from the control flour which had been doughed up with 13 c. c. of aqueous chloropicrin solution.

The most notable effects on the gluten are, however, not evident in the numerical values which have been given. These effects are physical. The glutens prepared from the untreated flour are elastic and tenacious, while those washed from treated flours lack these properties so desirable in gluten.

It was suggested that the chloropicrin might change the hydrogen-ion concentration of the doughs from which the glutens were washed; and since it was known that changes in hydrogen-ion concentration effect a dispersion of the gluten, this factor was investigated. It was found, however, that chloropicrin effected no significant change in hydrogen-ion concentration of dough suspensions, hence the chloropicrin must owe its deleterious action on the gluten to some other property.

These effects of the chloropicrin on the properties of the gluten were shown by other methods. Twenty-gram quantities of the flours were washed first with 1,000 c. c. and then with 500 c. c. of distilled water, and the viscosity determined in the manner outlined by Gortner and Sharp (14). The control flour at this concentration showed a viscosity of 197° McMichael. Two treated flours gave values of 146° and 151°, respectively. This again bears out the fact that the chloropicrin affects the ability of the gluten of flour to imbibe water from a weak acid solution.

In order to measure any effects on the elasticity or tenacity of the gluten or rather of the dough, certain experiments were carried out with the Chopin (11) extensimeter. In the use of this apparatus, 300 gms. of flour is made into a dough with 5 per cent sodium-chloride solution. Portions of this dough are pressed into a thin layer between metal plates. This thin sheet of dough is held in place so that air can be introduced from below. Under air pressure, the dough can be blown into a thin membrane which takes the shape of a bubble. The size of the bubble so formed indicates certain properties of elasticity of the dough.

Samples of treated and untreated flour were tested in this manner. The untreated flour gave a value of 16.26. Two samples of the same flour fumigated with chloropicrin gave values of 13.8 and 13.2, respectively.

From the data, it is evident that chloropicrin affects the properties of gluten to a marked degree. However, the flours were given drastic fumigation treatment, probably much more severe than they would receive in a mill or elevator. It appears that the chloropicrin is retained rather tenaciously by the flour. This is shown by the fact that when treated flour having no odor of chloropicrin is mixed with water the chloropicrin is set free as indicated both by odor and by its effect on the eyes. Probably the best method to determine whether flour has been treated with chloropicrin is to taste it. A sharp, biting taste will indicate the presence of chloropicrin even where any analytical method known would probably give negative results.

BREAD-MAKING EXPERIMENTS

Attention will now be directed to the effects of chloropicrin on flour and yeast in mixture as used in bread making. The baking procedure is that recommended by Bailey (2), 450 gms. flour being used in the preparation of the dough, one-third of which is subsequently placed in the expansimeter. Table IV gives the results and Figure 3 shows sections of the bread so prepared. The bread shown in Figure 3 will be referred to as the series of July 13, 1923. The No. 1 bread was made from the untreated control flour.

A portion of the same flour from which gluten No. 3 in Table III had been prepared was used in this baking series. It had, however, been lying spread out in a thin layer for two weeks, and glens prepared from it at the end of this additional exposure to the atmosphere compared quite favorably with those prepared from the untreated flour. Also, the bread baked from this excessively treated flour compared favorably with that baked from the untreated flour. These observations seemed to indicate that treated flours, if given sufficient aeration or exposure to the atmosphere, may recover from the effects of the chloropicrin treatment. These effects, there-

fore, must be due to the presence of the chloropicrin in the flour. A section of the bread baked from this flour is shown in Figure 3, No. 2.

TABLE IV.—*Baking tests on chloropicrin-treated flours, series of July 13, 1923*

No.	Treatment	Time to the oven	Loaf volume	Expansion meter
		Minutes	C. c.	C. c.
1	Control.....	295	1,720	860
	{Same flour Aug. 15, 1923.....	280	1,580	930
2	{Treated with chloropicrin and aerated.....	330	1,750	830
	{Same flour, Aug. 15, 1923.....	280	1,520	940
3	{Treated with chloropicrin and aerated to less degree than No. 2.....	325	1,440	650
	{Same flour, Aug. 15, 1923.....	260	1,590	980
4	{Treated with chloropicrin and aerated to less degree than No. 3.....	385	600	200
	{Same flour, Aug. 15, 1923.....	265	1,520	730
5	10 c. c. aqueous chloropicrin added to the dough.....	305	1,780	750
6	20 c. c. aqueous chloropicrin added to the dough.....	365	1,500	700
7	30 c. c. aqueous chloropicrin added to the dough.....	365	750	310
8	Treated with chloropicrin and insufficiently aerated.....	365	600	280

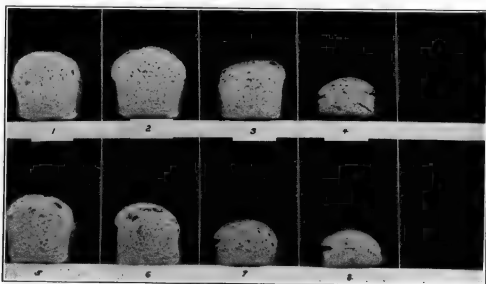


FIG. 3.—Baking tests on series of July 13, 1923: No. 1, control flour; Nos. 2, 3, 4, 8, chloropicrin-treated flours, arranged in order of degree of aeration, No. 2 having been subjected to the most aeration; Nos. 5, 6, 7 c. c. doughs treated with 10, 20, and 30 c. c. of aqueous chloropicrin solution, respectively

Considerable difficulty, however, attended the removal of the chloropicrin from the flour, as is indicated by the following experiment. A sack of the control flour (about 2 kilograms) was suspended over an excess of liquid chloropicrin, and removed after three weeks' exposure to the vapors. This sack of flour was allowed to remain out of doors for 16 hours. A portion of this flour was placed in a Mason jar, which was then stoppered, and another portion was spread out in a thin layer and left exposed to the atmosphere for 24 hours. At the end of the 24-hour period bread was prepared from portions of each of these flours. These breads are shown in Figure 3, Nos. 3 and 4, No. 3 being the one which was given the greater exposure. The bread prepared from both these flours was considerably inferior to that prepared from the control flour both as regards volume and texture. The flour which was given the greater chance to allow the chloropicrin to evaporate gave the more desirable loaf of bread.

In the preparation of the bread represented by Nos. 5, 6, and 7 in Figure 3, 10, 20, and 30 c. c. of aqueous chloropicrin solution were added, respectively. As the quantity of added chloropicrin increased, the volume of the resultant loaf of bread decreased. Thus, bread No. 7, in the preparation of which 30 c. c. of aqueous chloropicrin solution was used, attained a volume of only 750 c. c. as compared with 1,720 c. c. for the control flour. The addition of 10 c. c. of the chloropicrin solution did not markedly affect the volume of the loaf, but it did lower the expansimeter reading to 750 c. c. as compared with 860 for the control.

No. 8 shows the bread prepared from the control flour fumigated for 16 hours and aerated with an electric fan for 4 hours. The effects of the chloropicrin on the bread are even more marked than in the bread to which 30 c. c. of aqueous chloropicrin had been added. The volume of the bread prepared from this fumigated flour was 600 c. c., and the expansimeter reading 280 c. c.

The data which has just been presented indicate in a definite manner the detrimental effects which small quantities of chloropicrin have on the ability of flour to produce a good loaf of bread. Even in cases in which a fairly good loaf of bread was produced, the effect of the chloropicrin in retarding fermentation, i. e., in increasing the fermentation period, may be noted. In case of bread No. 7, in which 30 c. c. of the chloropicrin solution (50 mgms. of liquid chloropicrin) was used, activity of the yeast was almost stopped. The effects were even more marked in Nos. 4 and 8, in which the treated flours were not sufficiently aerated. In none of these treated flours was there olfactory or lachrymal indication of the presence of chloropicrin. On preparing the dough, however, the presence of the fumigant could not be doubted, the wetting of the flour setting free the chloropicrin. Tasting resulted in the same conclusion, that chloropicrin was present.

It was thought that if the flours were given sufficient time the chloropicrin would vaporize and leave them unharmed. Evidence for this belief is found in the fact that flours which at one time gave glutens of very poor quality seemed to recuperate and later give glutens of more desirable properties. Flours Nos. 1, 2, 3, and 4 of the series of July 13 were stored in cloth sacks until August 15, when bread was again made from them. Nos. 1, 2, 3, and 4 refer to portions of the same flours as were used on July 13, 1923, the first being the check flour and the last three being flours treated with chloropicrin. Table IV and Figure 4 show the results.

These data show complete recovery of the flours from the effects of chloropicrin, as far as the baking qualities were concerned. Flour No. 3, on July 13, gave a volume of 1,440 c. c. and an expansimeter reading of 650 c. c., as compared with 1,590 c. c. and 980 c. c., respectively, one month later; while for flour No. 4 the recovery is even more evident. On July 13 this flour gave a loaf volume of 600 c. c. and an expansimeter reading of 200 c. c.; on August 15, a portion of the same flour gave a loaf volume of 1,520 c. c., and an expansimeter reading of 730.

EFFECTS OF CHLOROPICRIN ON WHEAT

A series of experiments with fumigated wheats was carried on. For purposes of comparison, the wheat which was to be used in the fumiga-

tion studies was milled into flour, and bread was made from the flour. A section of the bread baked from this flour from untreated wheat is designated No. 5 in Figure 4. In Table V and Figure 4 the reference numbers indicate wheats which were treated in different ways.

TABLE V.—*Baking tests on flours milled from treated wheats, series of August 15, 1923*

No.	Treatment	Time to oven	Volume of loaf	Expansimeter
		Minutes	C. c.	C. c.
5	Control	280	1,620	860
6	Treated with chloropicrin vapor and aerated	270	1,480	900
7	Excess of chloropicrin vapor and aerated	300	750	200
8	Excess of chloropicrin vapor held 7 days	300	825	240
9	Treated with CS ₂ vapors and held 16 hours	245	1,200	700



FIG. 4.—Baking tests on series of August 15, 1923. No. 1, control flour; Nos. 2, 3, 4, baked from portions of the same lots of flour as were used for the same numbers in the July 13 series; No. 5, control flour; Nos. 6, 7, 8, fumigated wheats given various degrees of aeration; No. 9, a CS₂ treated flour

Five thousand grams of the wheat was placed in a jar of 8-liter capacity and exposed to the vapors from 0.38 c. c. of chloropicrin. After a period of one week, the wheat was removed from the jar, put in a burlap bag, and hung in the mill. Sixteen hours later one-half of it was milled into flour, and on the following day bread was baked from this flour. This bread was inferior to that prepared from flour milled from untreated wheat, both as regards loaf volume and texture. The volume of the bread from the treated-wheat flour was 1,480 c. c. as compared with 1,620 c. c. for the bread made from the untreated-wheat flour.

Another 5,000-gram portion of this wheat was placed in an 8-liter jar and exposed to the vapors from an excess of liquid chloropicrin for a period of one week. It was then removed from the jar, placed in a sack, and 16 hours later one-half of it was milled into flour. On the following day, the flour was baked into bread. A similar portion of wheat was treated in the same way, except that it was milled into flour one week after it had been removed from exposure to the chloropicrin. Sections of the bread prepared from these treated-

wheat flours are designated in Figure 4 as Nos. 7 and 8, respectively. Bread No. 7 gave a loaf volume of 750 c. c., and an expansimeter reading of 200; while bread No. 8, prepared from treated wheat, which had been given a greater time to recover from the chloropicrin treatment, gave a volume of 825 c. c., and an expansimeter reading of 240 c. c. Since a tendency of the treated wheats to recover from the chloropicrin treatment was noted, portions of these wheats were retained and used in later experiments.

The data which have been given showed that even with the extensive manipulation and aeration of the wheat and its products during the milling process, the chloropicrin was not sufficiently well removed from the flour to insure good results in the bread made from them. Glutens washed from flours milled from these treated wheats showed the same inability to hold water, and the same tendency to be inelastic and noncoherent as was shown by chloropicrin-treated flours.

In connection with the detection of chloropicrin in wheat, the remarkable penetrating power of the fumigant was observed. When a few chloropicrin-treated wheat grains were placed in the mouth, no taste of chloropicrin was noted. If, however, these grains were broken up by the teeth the presence of chloropicrin was unquestionably indicated.

The presence of bread No. 9 in the August 15 series was only incidental. This bread was prepared from the control flour which had been treated with carbon disulphide and then removed from the container 16 hours before it was baked into bread. The volume of this loaf of bread was only 1,200 c. c. as compared with 1,580 c. c. for the untreated control flour. That carbon disulphide may affect the bread made from flours fumigated with it appears to be quite certain.

It has already been shown that flours can recover from the effects of the chloropicrin treatment if exposed to the atmosphere for a sufficient time. It was therefore reasonable to expect the wheats to recover in like manner. Table VI and Figure 5 give the results of experiments with treated wheats which were held in storage for a period of time before being milled. The series of experiments shown in Figure 5 will be referred to as the series of September 7, 1923. No. 1 shows a section of the bread prepared from the same control flour as has been used as a standard throughout this work, and No. 5 shows the bread made from the flour milled from the untreated wheat.

The breads designated as Nos. 2, 3, and 4 were prepared from flours milled from wheat exposed to chloropicrin vapors for 24 hours. The wheats were given 10 days to recover before they were milled into flour. Even wheat No. 4, which was treated so that liquid chloropicrin was present throughout the fumigation period, gave a loaf of bread the volume of which was 1,560 c. c. This compares quite favorably with the loaf volume of 1,620 c. c. shown by the control-wheat bread. Bread No. 3, treated with the vapors from 0.38 c. c. of chloropicrin, had a loaf volume identical with that of the control-wheat bread.

Breads Nos. 6, 7, and 8 were prepared from flour milled from portions of the same wheats as were used in the series of August 15. The quality of the bread baked on September 7 indicates that the wheat, even that treated with excess of chloropicrin, has almost entirely recovered from the effects of the chloropicrin treatment.

Bread No. 6 had a loaf volume of 1,480 c. c. on August 15 and a volume of 1,810 c. c. on September 7; bread No. 7 had a volume of 750 c. c. on August 15 and a volume of 1,700 c. c. on September 7, and bread No. 8 had increased from a volume of 825 c. c. on August 15 to 1,560 c. c. on September 7.

TABLE VI.—Baking tests on flours milled from treated wheats, series of September 7, 1923

No.	Treatment	Time to oven	Volume of loaf	Expansimeter
		Minutes	C. c.	C. c.
1	Control.....	335	1,560	760
2	Treated wheat given 10 days to recover before being milled.....	335	1,600	800
3	The same, 0.38 c. c. chloropicrin.....	330	1,620	830
4	The same, excess chloropicrin.....	325	1,560	850
5	Control wheat.....	335	1,620	760
6	Same flour as No. 6, Aug. 15.....	350	1,810	740
7	Same flour as No. 7, Aug. 15.....	345	1,700	740
8	Same flour as No. 8, Aug. 15.....	335	1,560	820

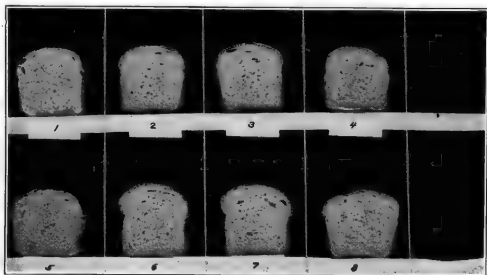


FIG. 5.—Baking tests on series of September 7, 1923: No. 1, control flour; Nos. 2, 3, 4, wheats given 10 days to recover from fumigation before being milled; No. 5, flour milled from control wheat; Nos. 6, 7, 8, wheats held over from August 15 series and milled for this baking

The data obtained from these experiments again indicate the ability of the wheat to recover from the effects of the chloropicrin treatment. The recovery is more rapid in the case of wheat than it is with flour. The explanation probably lies in the greater absorptive power of the finely divided flour for chloropicrin as compared with that of the wheat.

CONCLUSIONS

Chloropicrin is highly toxic to insects.

The factors of time and temperature bear an inverse ratio to each other when concentration varies from 1 gm. to 125 gms. per cubic meter.

When concentration is constant, the time required to kill bears a linear relationship to temperature, and this relationship continues down to 0° C.

Under practical conditions, it is necessary to increase the volatility by atomizing the chloropicrin in order that a lethal concentration may be built up in the atmosphere before the loss due to leakage so reduces the amount of chloropicrin present that a lethal concentration can never be attained.

When the spray of chloropicrin is directed toward the infested areas, the insects are exposed to a saturated atmosphere while the entire volume of the room very quickly attains a lethal concentration.

When chloropicrin is present in the flour, deleterious effects are noted in the bread produced from such flour.

These effects are due to the ability of the chloropicrin to retard fermentation and to affect in a harmful manner the physical condition of the gluten-forming proteins.

When flours or wheats treated with chloropicrin are exposed to the atmosphere for sufficient time, the chloropicrin disappears from them.

Fumigated flours given proper exposure to the atmosphere show complete recovery from the chloropicrin treatment.

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OBSERVATIONS ON THE HIBERNATION OF GROUND SQUIRRELS¹

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INTRODUCTION

While making observations on the life history of the Columbian ground squirrel (*Citellus columbianus columbianus*) difficulty was experienced in obtaining data on the hibernation. Information on this subterranean phase of the animal's life cycle was obtained, however, by patient and long-continued search and excavation in the field. But there still remained many hidden and obscure points, especially in regard to the day-by-day activity of the animal while in the state of aestivation and hibernation, which could be learned only by observations repeated continuously during the entire period of coma.

To make these observations possible it was necessary to have the animals under partial and absolute control. This was accomplished by carefully planned and properly constructed yards for observations of a semirestricted nature and a cabin and hibernation cellar for closer and more detailed observations. For the purpose of the former, the undisturbed dens of wild squirrels were securely fenced in.² For the latter, a cabin (fig. 1) was constructed with which were connected small yards and beneath which was a cellar furnished with small compartments in which aestivating and hibernating squirrels could be placed and be easily observed, day or night, at the will of the observer. With this equipment, wild squirrels were obtained from the fields and the work was begun, with the following results.

As might have been expected, the sudden transfer from wild to captive life caused the squirrels to break the regularity of their life routine, and it was usually some weeks before they overcame their fear of the new surroundings and went into the comatose condition. Indeed, if food were continually furnished them they would sometimes remain awake during the entire winter.

To induce them to go into hibernation, food of fattening properties, such as they were accustomed to obtain under wild conditions, was approximated in the form of a sheaf of wheat or oats and the seeds of sunflowers. Moisture was given in the form of carrots and apples, of both of which they are very fond.

Besides those placed in the hibernation cabin, others were confined in the yards of a nearby brood cabin used for determining other phases of the life history. Here, as in the former place of confinement, they usually dug small dens in the accompanying yards and there transferred the nest. This was desirable, as it removed them from the effect of the unnatural condition of midday heat in late summer and

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² SHAW, W. T. A LIFE HISTORY PROBLEM AND A MEANS FOR ITS SOLUTION. Jour. Mammal. 6:157-162, illus. 1925.

also from the disturbing conditions attending feeding and the care of the yards. In the hibernation cabin in many cases they went into hibernation in the nests provided for them in the cool underground boxes. In fact, these seemed so well adapted for them that in the summer of 1915 a squirrel in one of the adjoining yards took up his abode in one of these nests which he had succeeded in reaching through a defective partition.

Those squirrels which had gone into hibernation in the earth of their respective small yards were dug out of their nests, weighed, and transferred to the nest boxes in the hibernation cabin. Here they were visited almost daily and their condition and weekly weight noted.

The records of females Nos. 7 and 8 and of males Nos. 8 and 9 are given here, showing the activities and inactivities as they oc-

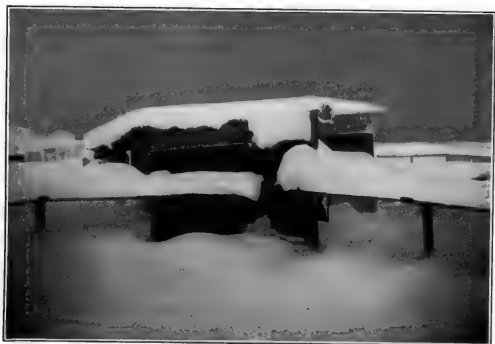


FIG. 1.—Hibernation cabin in winter. Sometimes, in the dead of winter, the cabins became all but covered with snow. Then were presented true conditions of hibernation. Body temperatures were taken, showing a fall in temperature to 40° F., at which time the squirrels were but little higher in temperature than their surroundings.

curred and giving the weights from time to time. Also the record of a Townsend squirrel is given as illustrative of some points not so well shown by the more timid Columbian.

HIBERNATION RECORDS

The hibernation records of female 8, showing her behavior during the winter, are given as follows: Female 8 was captured August 16, 1911, while in wild aestivation. On that date she weighed 334 grams. On being placed in yard 11, she awoke from aestivation and remained awake until September 18. Meantime, on August 21, she began the construction of a den in the earth of the outer yard. On August 23 she moved all of her nest from the box out into the newly constructed burrow in the outer yard. From September 18 to November 10 she was allowed to remain in undisturbed hibernation. On November 11 she was dug from her nest and placed in the box of yard 11.

On this date she was entirely in hibernation, but showed signs of wakening. She was observed almost daily from November 12, 1911, to March 13, 1912. Her subsequent behavior is as follows:

She was awake and warm on November 12, 13, 23; December 7; February 23, 24; March 6, 7, 8, and 12. The hibernation period ended March 12.

Her weights were as follows:

	Grams		Grams
Nov. 18.....	466	Jan. 20.....	413
Dec. 2.....	450	Jan. 27.....	409
Dec. 9.....	441	Feb. 4.....	408
Dec. 16.....	441	Feb. 17.....	399
Dec. 30.....	425	Feb. 25.....	385
Jan. 6.....	420	Mar. 2.....	387
Jan. 13.....	420	Mar. 9.....	370

This squirrel was awake on five different occasions during the winter. She lost 90 grams in weight between November 18, 1911, and March 9, 1912. She was deprived of all food from November 10 to March 13.

INTERMITTENT AWAKENING FROM HIBERNATION

As was shown by female 8, squirrels were wakening at intervals during the winter. Very careful daily observations relative to their condition revealed this fact. Males 8, 9; female 7, and *Citellus townsendi*, male 5, all showed the same characteristic of interrupted hibernation.

That they can pass in and out of the hibernation state rather easily is shown by *Citellus townsendi* No. 3, which, on November 8, was awake, November 9, in hibernation, and November 10 awake again. It was noted while making the daily observations that the squirrels would show signs of life and become somewhat warm occasionally without actually awakening.

OBSERVATIONS ON AN AWAKENING SQUIRREL

These animals are very lifeless in appearance and form when in the condition of hibernation. The eyes are very sunken and tightly closed. The mouth and lips are formless as in death. No sign of life remains, save a possible slow movement over the flanks, during which period the hairs of the region are slowly caused to bristle in erection. One in profound hibernation was removed to a warm room, and as she began slowly to show signs of life the following observations were made of her:

At 3.20—Rhythmical thoracic respiration movements were noticeable. Mouth was opened slightly.

At 3.32—Squirrel began to rub paws over her nose. Eyelids began to twitch. Body began to straighten out, and the front legs began to work in a writhing motion, from side to side, over the chest, the paws being held together about the middle of the belly.

At 3.40.—This action was speeded up. The motion was fairly regular, suggesting the beating of the heart (the alternate contraction of auricle and ventricle).

At 3.50.—Change took place. Up to this time very little or no movement of the hind legs and the hinder part of the body, but now the hind legs began to move. At this time, owing to the lateness of the day and failing photographic light, the squirrel was warmed by artificial heat.

At. 3.55.—Regular respiratory movements commenced, accompanied by muscular movements of legs and back. Animal straightened out. Hitherto she had been lying on her side, trembling like a new-born lamb, and apparently unconscious and helpless. Now her front limbs were shaking like one with the ague, while the hind limbs were perfectly still.

At 4.05.—She was gasping for breath.

At 4.10.—Violent shaking of the front paws, with a straightening out of the body. There was still little motion of the hind limbs.

At 4.22.—She was able to maintain a position on her belly, attempting to crawl. Eyes still shut.

At 4.25.—Rapid heart beat noticeable. For the first time the muscles began to be resistant. There was quite a tenacious grip in the toes. The tail was controlled by muscular movements. She responded nervously when touched.

At 4.33.—Squirrel was very warm and comfortable, and well able to crawl about. It was now difficult to keep her in front of the camera.

One very noticeable feature was the very great acceleration in the awakening process, beginning at 4.25 and terminating with the almost completely wide awake condition at 4.50.

Three other squirrels were studied, and in each case similar observations were made. In all of these it was noticed that the posterior part of the body remained inactive and lifeless much longer than the anterior. These observations were made independently, but later it was noted that a French experimenter, Marès,³ showed by injecting ground squirrels that the circulation in the posterior part of the body was largely suspended during hibernation and that this part of the body was the last to come from that condition.

That they regain fleetness and acutenss of their senses in a short time is shown by the following incident: On November 18 a squirrel was dug from hibernation two days before it was taken back to its den to be photographed. Suddenly it made a dash for liberty and darted into a very obscure hole beside a fence post, about 3 rods from its den, seeming to be familiar with the surrounding territory and in keen possession of its senses.

Interesting observations were made during the winter in regard to daily weight losses and the duration of hibernation. The records are given in Table I. This table shows a rather constant loss of about 1 gram a day for both the Columbian and Townsend squirrels.

TABLE I.—Weight losses, in grams, of five squirrels during winter hibernation

Species	Weight when going into hibernation	Weight when coming out of hibernation	Total weight lost	Duration of hibernation in days	Amount lost per day in grams
C. c. columbianus male No. 8.....	592	530	62	63	0.98
C. c. columbianus male No. 9.....	445	394	51	28	1.8
C. c. columbianus female No. 7.....	414	273	141	77	1.8
C. c. columbianus female No. 8.....	500	370	130	119	1.09
C. townsendi male No. 5.....	280	221	59	56	1.05

³ MARÈS, F. EXPÉRIENCES SUR L'HIBERNATION DES MAMMIFÈRES. (Mémoire) Compt. Rend. Soc. Biol. [Paris] (sér. 9, t. 4) 44: 313-320. 1892.

After awakening from hibernation the squirrels began to take on weight, as is shown by the record of female 8. On March 9, three days before the hibernation ended, she weighed 370 grams, on May 16, she weighed 411 grams, having gained 40 grams in 64 days or 0.62 gram per day, a little more than half the daily loss in the winter. There was a greater gain shown by female 4. On coming from hibernation, probably March 14, she weighed 301 grams on March 19; on May 16 she weighed 422 grams, having gained 121 grams in 58 days, or a little more than 2 grams per day. Of course, the weight on May 16 might have been increased by bulky food. Immature male 6 increased 55 grams in weight between March 30 and May 24; immature female 10 increased 86 grams between April 4 and May 12; immature male 11 increased 28 grams between March 30 and May 24.

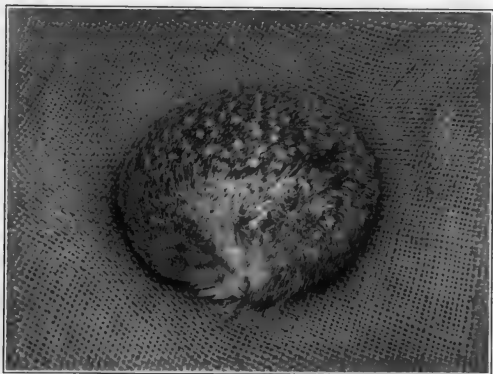


FIG. 2.—A Townsend ground squirrel in hibernation. This squirrel, brought from low elevation of 1,471 feet, went into aestivation at the remarkably early date of June 14. After an almost continuous sleep of eight months, he appeared as in Figure 3

DURATION OF HIBERNATION IN THE HIBERNATION CABIN

A very remarkable hibernation record was that of the *Citellus townsendi* male 5. For a few days prior to June 14, 1912, he had not been eating regularly and had been plugging up the cracks in the nest box and the hole in the chute leading to the yard. On June 14 he was found in aestivation (fig. 2). He passed through the usual hibernation. On January 31, 1913, he showed signs of having been awake, as he had torn down part of the plug in the exit shaft, which had been untouched since being put in by him in June. He was still in hibernation February 13, having been in a state of aestivation and hibernation for a period of 244 days or, roughly speaking, 8 months. It is possible he would have ended his hibernation in January had he been at liberty, but finding the exit

of his box closed he acted philosophically and postponed starvation by returning again to hibernation. On February 14 it was found that he had been gnawing in an effort to get out, so he was given his liberty. On this date he was fully awake and came out into the snowy yard and ate some carrot (fig. 3).

On coming from hibernation permanently they soon repair their nest. This is done by piling up the material in a neat, fresh, warm-looking structure suitable for an active life.

DURATION OF HIBERNATION IN THE LARGE YARDS

In the winter the large dens of the yards were used for hibernation studies. It was found that the duration of hibernation for two males was 169 and 131 days; for 4 females, 190, 178, 178, and 179



FIG. 3.—Yard observations. In the cellar, it was possible to observe squirrels daily the winter through, thus enabling comparison of these conditions with field conditions. This squirrel (a Townsend) slept almost continuously for eight months. He awoke at about the normal time, but, not finding food, went back to sleep.

days; for 2 young, 209 and 233 days. Of course, conditions were not normal, as in the fields, and the squirrels were slow in going into hibernation. The young, being tamer, went first. As in the fields, the duration of hibernation is greater among the females than among the males, and in one case the female returned from hibernation 15 days later than the male. One male lost 123 grams in 169 days or 0.7 gram per day.

TEMPERATURE OF BOXES

Nine daily records taken between August 14 and September 9, 1912, showed that the summer temperature of the boxes was much more constant and favorable to aestivation, varying only 7 degrees, against a 35-degree range of air temperature and averaging 15 degrees lower than the air. A hibernating squirrel can resist a

temperature 4° to 6° F. below freezing about its nest. On February 3, 1914, the box temperature where the squirrels were kept was 30.5° F., resulting in no harm to the hibernating squirrels. From this one can understand why it is that young squirrels risk the danger from frost when hibernating only 6 or 7 inches below the surface of the ground.⁴ It was noted also in the hibernarium that the squirrels would come out of the hibernation condition if the boxes containing them went much below the freezing point. Of course, the nest material would assist in keeping the temperature up (fig. 1).

BODY TEMPERATURE OF HIBERNATING SQUIRRELS

The body temperature of squirrels in hibernation was taken. At first this was reached approximately by placing the thermometer against the ventral side of the squirrel. Later, rectal temperatures were taken. The results are given in Table II. From this table it is seen that the average body temperature of three hibernating squirrels tested by the rectal method is 42.3° F., or about 56° below normal. It is noteworthy that in the case of the squirrel tested February 12 her temperature rose from 38° F. to normal 98° in four hours.

TABLE II.—*Body temperatures of hibernating squirrels, in degrees Fahrenheit*

Date	Kind of test	Temper- ature of nest box	Temper- ature of squirrel	Dura- tion of test
				<i>Min.</i>
Jan. 20, 1912.....	Contact.....	38.5	41.0	-----
Mar. 1, 1912.....	do.....	34.0	38.1	-----
Feb. 3, 1914.....	Rectal.....	30.5	49.0	18
Feb. 12, 1914.....	do.....	36.0	38.0	6
Feb. 16, 1914.....	do.....	-----	40.0	15

RELATION OF FEEDING TO AESTIVATION AND HIBERNATION

It was noticeable that squirrels preparing for aestivation or coming from hibernation used very little food, and this was taken at intervals of some days. For example, in August a Columbian ground squirrel was observed to have an interval of seven days between times of eating. On August 25 it partook of a very little food. This seemed to be its last meal before going into hibernation. A Townsend squirrel did not eat much for several days before going into aestivation.

That they need not eat during hibernation is shown by a Columbian under observation. It was given no food from November 1, 1911, to March 13, 1912. When they were given food while awake it seemed to interfere with the regularity of their hibernation and even to keep them out of this condition altogether. In the case of Columbian female 7, taken from the hibernation condition on November 11, 1911, she afterwards awoke and was fed November 14. She remained awake for some days, but went into hibernation November 20. She ate again December 14, and was in hibernation again on December 16. She ate once more January 1, 1912, and went into hibernation January 2. Again on January 17 she ate and went back into hibernation the following day. On January 29 she awoke and ate and did not go back into hibernation.

⁴ SHAW, W. T. THE HIBERNATION OF THE COLUMBIAN GROUND SQUIRREL. *Canad. Field-Nat.* 39 56-61, 79-82, illus. 1925.

Another case of feeding was that of male 9, given food on December 31, 1911. He was awake and ate on January 11 and 14, after which he remained awake. *Citellus townsendi* male 5 ate sparingly the first day out of hibernation.

For squirrels to consume food at all in the winter is not normal. Occasionally in the yards a squirrel would not go into hibernation, but remained warm and active, consuming very little food. On December 11, 1913, a squirrel was tested for the amount of food consumed in a day and it was found that it ate only 8 grams of carrot and sunflower seed. It showed a preference for the seeds. On December 21 it ate 4 grams of carrot and 1 gram of sunflower seed, making 5 grams in all.



FIG. 4.—In the yards, some of the squirrels, captured late in the season and being very wild, would not enter into hibernation, and seemed to take very little food during the entire winter. In order to make observations regarding the necessity of food in winter, though awake, this squirrel was placed in hibernarium box which had been completely cleaned so that no food should remain. A warm nest of excelsior and cotton batting was provided. The squirrel was observed from day to day. During all the time of his fasting he seemed to be in good condition and wide awake. At the end of the sixteenth day without food he went into hibernation, as shown in the figure

That they can get along without food at this period is shown by the following observation: One of these squirrels, refusing to go into hibernation, was deprived of food for 16 days, during which time it remained awake, warm and lively. On the seventeenth day it went into hibernation, remaining in this condition for six days (fig. 4). At the beginning of the test it weighed 495 grams. At the end of the test it weighed only 358 grams. On awakening, it ate and continued its existence as if nothing had happened (fig. 5). That aestivation and hibernation are offsets to starvation is evident.

APPEARANCE OF A HIBERNATING SQUIRREL

Throughout all the work of hibernation it was observed that the squirrels, especially during the last of the season, became very lifeless. They took the vertical position of the squirrels shown in Figure 4, a position maintained only in a well-packed nest, and dropped to the temperature already described. They appeared less rigid during

the first few days of aestivation, as is shown by a Townsend squirrel in Figure 6. Their eyes were sunken very deeply into the orbits, and their lips became very shapeless and deathlike. Occasionally,



FIG. 5.—Same squirrel as shown in Figure 4. After going into hibernation on the sixteenth day of fasting, he slept for about six days, when he awoke and was given food. Later he was branded and turned in one of the larger outer yards where he ran all summer, regaining his normal weight



FIG. 6.—Observations in the yards have given interesting testimony on the manner in which aestivation comes over the squirrels. During the few days previous to going into the comatose condition, they gradually cease eating. Then, as in the case of the Townsend squirrel shown here, they go into torpor. They do not always curl up tightly at first, and they may awaken after a day or so. At this time they get back their normal temperature, which they may retain for a short time before going back to sleep. Later in the winter, however, they appear extremely torpid, and at times it is difficult to believe they are really alive, as shown in Figure 2

after long watching, one would see a movement pass across the flank, resembling in slowness that of the peristalsis movement shown by a recently chloroformed mammal in the laboratory. From this condition they might transform in a few hours to the state of very live and warm squirrels.

THE VALUE OF CALCIUM PHOSPHATE AS A SUPPLEMENT TO THE RATION OF DAIRY COWS¹

By J. B. LINDSEY, *Vice Director and Chemist*, and J. G. ARCHIBALD,² *Assistant Professor of Research Chemistry, Massachusetts Agricultural Experiment Station*

INTRODUCTION

The mineral requirements of the animal system constitute a phase of the science of nutrition which has only within recent years attained any degree of prominence. From the time when Voit and his co-workers first established on a firm basis the nutritive function of those major constituents of foodstuffs—protein, carbohydrates, and fats—up until as late as the close of the first decade of the present century, energy values and nutritive ratios were the only standards by which food values were measured. Because of the prominent place which these factors occupy in any rational system of nutrition, it was only natural, and in one sense right, that emphasis should be placed on them to the exclusion of minor factors whose significance and importance either were not realized at all, or, if realized, were but imperfectly understood.

One of these minor factors was an adequate supply, both as to quantity and quality, of the mineral elements necessary for the proper functioning of the animal system. It is true, of course, that the necessity of the presence of these inorganic constituents in the diet was long ago recognized and their functions in the body understood, but the general conception was that an otherwise normal diet supplied a sufficiency of mineral matter. It was not realized that there may be times when the supply of mineral matter in the ration becomes a limiting factor in the functional well-being of the animal. Armsby (1, p. 332, 333)³ sums up the situation in the following terse sentences: "Most feeding stuffs, however, and particularly the mixed rations of farm animals, contain what appears at first sight to be much larger amounts of ash ingredients than the body requires." As a consequence the idea became somewhat prevalent "that rations adequate in other respects may be assumed to contain a sufficiency of ash ingredients. This is doubtless true of animals living in a state of nature, but it is a questionable assumption under the artificial conditions to which many farm animals are subjected."

That animals may at times suffer from a shortage of mineral matter in their rations is now well established. The present status of our knowledge on this point, and on the whole problem of mineral metabolism in farm animals, is due largely to several important researches made in recent years, and can best be outlined by a brief review and summary of these researches. In all the researches to be mentioned, the emphasis has been put largely on the metabolism of calcium and phosphorus, and especially on the former. There are

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² This project was planned by the undersigned with the cooperation of Mr. Archibald. The latter supervised the execution of the experiment, holding frequent conferences with me as it progressed. Owing to my many duties, it became necessary for him to prepare this paper for publication.—J. B. Lindsey.

³ Reference is made by number (italic) to "Literature cited," p. 790.

two reasons for this: (1) the important part played by these elements in the animal system, and (2) rations are more likely to be deficient in them than in any other mineral element.

Forbes (4, 5, 6, 8, 9,) and his coworkers at the Ohio Agricultural Experiment Station conducted a very elaborate and comprehensive series of metabolism experiments with cows in different stages of lactation. They found that high-producing cows in the earlier stages of lactation, even when fed on rations rich in calcium and phosphorus, invariably showed negative calcium balances and generally had negative phosphorus balances. This negative balance persisted until the later stages of lactation. When the milk flow had dropped to about 10 pounds daily the animals began to show positive balances of calcium and phosphorus, and from that time until calving the intake of these elements was greater than their rate of excretion. In other words, the losses of early and middle lactation were made good by storage during the later stages of lactation and in the dry period. Forbes considers this tendency on the part of milking cows to excrete their body reserves of calcium and phosphorus to be due to the intensification by breeding of the function of milk secretion to a point which is beyond the animal's ability to assimilate these elements from her feed.

As a result of his studies, Forbes recommends that cows be fed a ration naturally rich in calcium and phosphorus, i. e., one containing legume hay as a roughage, which is nothing more than a matter of good dairy practice. In addition he recommends a dry period of six to eight weeks, during which time the cow is to be fed liberally on a mineral-rich ration in order that she may make good the depleted mineral reserves of her body. He has also studied the possibility of supplying calcium in the form of mineral supplements, and his conclusion is that "it is not clear that supplemental calcium is utilized," although he suggests "for practical experimental purposes, the feeding of calcium phosphate in the form of steamed bone."

Forbes (3, 7) also conducted extensive experiments with swine on the value of mineral supplements in the ration. He found that pigs supplied with either precipitated calcium carbonate or special steamed bone had relatively dense and strong bones; that in pigs fed rock phosphate as a supplement the bones had about the same characteristics as those fed on a ration containing no mineral supplement, viz., relatively low breaking strength and density; that the hardest bones contained the highest proportions of calcium and total ash, while the softest bones contained the highest proportions of phosphorus and magnesium and relatively little calcium. He considered that the amounts of calcium, magnesium, and phosphorus in the bone are susceptible of much greater modification through the composition of the food than are their relative proportions. Special steamed bone proved to be the most satisfactory mineral supplement for swine of any he investigated.

Another prominent group of investigators in this field has been Hart and his colleagues at the Wisconsin Agricultural Experiment Station. Their earlier work was principally with swine. They demonstrated (13) that hogs are very sensitive to low ash content in their rations, that they can utilize inorganic phosphate to advantage, and that organic phosphorus (e. g. phytin) is no better than tricalcic phosphate for supplying the animal's needs for phosphorus.

Following up the investigation, they concluded (14) that calcium is the mineral element most likely to be deficient in ordinary rations and that swine should receive some form of supplemental calcium in addition to their grain ration.

Their work with cows on mineral metabolism has been the natural outgrowth of a most interesting series of experiments in which it was strikingly demonstrated that rations from different sources have a decidedly different biological value, regardless of how similar they may be with respect to digestible protein and net energy. In the case of rations restricted to the oat-plant, reproduction in cows was a failure, the calves being born either prematurely dead, or weak (16). When calcium salts, either carbonate, phosphate, or acetate, were added to the ration, a marked improvement in the reproductive function was noted.

Their more recent work has consisted of a series of mineral metabolism experiments with goats and cows, attention being centered on the assimilation of calcium (17). Their conclusion, as a result of four separate investigations, is that calcium assimilation is facilitated by the presence in the ration of an unidentified accessory substance which is present in green roughage and in dry roughage which has been carefully cured without exposure to direct sunlight, but not in dry roughage as ordinarily cured. They consider as a result of their work with goats (17) and dogs (23) that cod-liver oil contains the same substance. Their evidence on this latter point is strengthened by some work of McCollum et al. (21) who have demonstrated the existence of a fourth vitamin in cod-liver oil and other fats distinct from vitamin A and having to do with the metabolism of calcium.

Hart and his coworkers (17) also conclude that the ordinary grasses, even in their green state, do not furnish sufficient calcium to meet the needs of milking cows. The calcium must be supplied either in legume hay or as a mineral supplement, with the additional safeguard of some medium which carries the unidentified accessory substance essential to optimum utilization of the calcium.

Meigs, (22) of the United States Department of Agriculture, has also conducted a series of investigations on the subject of mineral matter for cows. His principal conclusion is essentially the same as that of Forbes, viz, that cows should be given a two-months' dry period, during which time they should be liberally fed on a ration rich in calcium and phosphorus. He noted a phenomenal increase in milk yield when cows were fed sodium phosphate with their grain ration on alternate days, and concluded that one of the principal reasons why cows often fail to maintain a high production record in successive years is because their rations are deficient in mineral matter.

The work of Welch (24), of Hart and Steenbock (15), and of Kalkus (19) on the relation of the iodine content of the ration to hairlessness and goiter in the newly born young of many classes of farm animals is another phase of the problem. The results of these three separate investigations have given quite conclusive proof that the prevalence of goiter and hairlessness in young animals in certain regions is due to improper functioning of the thyroid gland, caused by lack of iodine in the rations of the pregnant mothers, which can in turn be traced to lack of iodine in the soil and drinking water of

the region. The trouble can be largely corrected, if not altogether removed, by mixing small doses of potassium iodide with the feed of pregnant sows, ewes, does, cows, etc.

The foregoing researches constitute the chief contributions of recent years to the subject of the mineral requirements of farm animals. Although they have done much to advance the knowledge of the subject, there yet remain many phases of it to be investigated. The investigations with cows have for the most part been metabolism experiments, which of necessity can be conducted for only relatively short periods of time. Long-time experiments on the effect of feeding mineral supplements to milking cows are not recorded in the literature. Forbes, in his most recent paper on the subject (8), remarks that "The advantage to be derived from the feeding of mineral supplements seems to us doubtful, but . . . that the possibilities of benefit from so doing should be thoroughly investigated . . ." In a recent report of the subcommittee on animal nutrition of the National Research Council (10) occurs the following paragraph:

An important problem in this field is to determine, by carefully controlled, long-time feeding experiments with dairy cattle, under conditions of practice, the effect of differences of intake of mineral nutrients, especially as contained in leguminous as compared with gramineous roughage, and in mineral supplements, on growth and productive efficiency.

Such an investigation has been in progress at this station for the past two and one-half years and is still being conducted. This paper constitutes a report of progress on the project up to May 1, 1924.

SCOPE, METHODS, AND RESULTS OF THE INVESTIGATION

OUTLINE OF THE PROJECT

In brief, the essentials of the investigation have been as follows:

(1) Feeding to the station dairy herd of a ration as deficient in calcium and phosphorus as it has been possible to formulate from the roughages and concentrates available, but at the same time adequate in other respects.

(2) Division of the herd into two halves as nearly identical as the individuality of the animals would permit, and the addition to the grain ration of one of these groups (always the same group) of supplemental calcium phosphate in the form of a special steamed bone meal, the amount varying from 3 to 8 ounces daily, depending on the weight and milk yield of the cow.

(3) Observation of the effects of the mineral supplement upon:

(a) The general condition of the animals as apparent to the eye and as revealed by handling.

(b) Body weight.

(c) Milk production.

(d) Composition of the milk, especially its content of calcium and phosphorus.

(e) Reproduction, under which general heading have been observed:

(1) Recurrence of oestrus.

(2) Difficulties in getting cows to breed.

(3) Such abnormalities as abortions, retained placentas, etc.

(4) Condition of the calves at birth and subsequently.

METHODS OF EXPERIMENTATION

GENERAL PROCEDURE

The station herd is made up of high-grade Holsteins and Jerseys, and usually numbers, including young stock, about 18 to 20 head. At present there are 20 animals included in the experiment, 15 milk cows, 3 yearling heifers, and 2 eight-months-old heifers.⁴ There have of necessity been some changes in the herd since the experiment was started, but of the 19 individuals originally included, 13 are still in the herd. Since the commencement of the experiment in December, 1921, the milking herd has had an average annual production per cow of 8,200 pounds of milk, testing 4.52 per cent of fat. The herd is maintained for the most part by raising the heifer calves; only occasionally are any cows purchased.

All the herd is housed in a well-lighted, well-ventilated stable, and except in inclement weather receives daily exercise in adjacent yards. Up until last year all cows dry during the pasture season have been sent to pasture regularly for a period of four to six weeks. For reasons outlined later, this practice is no longer followed, only the young stock being pastured. All cows are given a two-months' dry period, during which time they are liberally fed. The aim is to have the cows freshen in the autumn whenever possible, but this is a matter which is difficult to regulate in practice. The cows are maintained on dry feed the year around, except for a period commencing about June 25 and ending about October 10, during which time they are fed such green soiling crops as oats, millet, sorghum, and fodder corn as a substitute for a portion of their hay. No ensilage or roots are fed. Running water is before the cows at all times, and salt is furnished to the extent of 0.75 per cent of the grain ration. The cows are fed and milked twice daily (at 7 a. m. and 5 p. m.); each cow's daily ration is carefully weighed out—the hay into 4-bushel baskets, the grain into suitable trays. The milk is weighed and accurate records kept of the production of each cow and the cost of the feed. Composite samples of every lot of hay and grain purchased are taken regularly, and the milk is sampled once each month for five consecutive days. The composite samples are analyzed as outlined later on in this paper.

All animals are weighed on the 1st and 2d of each month, and their rations adjusted according to their body weights and the amounts of milk they are giving. The standards for digestible nutrients employed in formulating and adjusting the rations are:]

For mature cows, Haecker's standard for the aged cow (12, p. 56-57).

For heifers that have had one calf, Haecker's standard for heifers (11, p. 107).

For heifers previous to their first calving, Armsby's standard for maintenance and growth in the dairy breeds (1, p. 713).

The standard adopted for calcium and phosphorus requirements of all the animals has been that proposed by Kellner (20, p. 618), founded in part on the observations of Henneberg (18, p. 53) and in part on Kellner's own work. This standard calls for a daily minimum of 100 grams of calcium oxide (CaO) and 50 grams of phosphoric acid (P₂O₅) for each 1,000 kg. of live weight, for maintenance only. In addition, for each 20 kg. of milk produced, there must be supplied

⁴ All heifer calves are included in the experiment as soon as old enough to subsist on hay and dry grain. The age varies with the calf from 5 to 7 months.

50 grams each of calcium oxide and phosphoric acid. Expressed as the elements (Ca and P)⁵ and on a pound basis the standard is as follows: A cow weighing 1,000 pounds requires for maintenance (daily), 0.07 pound calcium, 0.021 pound phosphorus; for each pound of milk produced, 0.002 pound calcium, 0.001 pound phosphorus.

The analytical data which have been employed in adjusting the rations to the standards have been very carefully compiled from a large number of analyses and digestion studies of the several materials fed. In the case of the mineral constituents of the various feeds, it was found at the start of the experiment that comparatively few analyses had been made, so a separate project⁶ was organized and the mineral constituents of a large number of samples of the roughages and concentrates fed were determined.

RATIONS FED

As already stated, the ration fed has designedly been as poor in mineral matter as a wise choice of feeds, other factors being considered, would permit. It has not been possible or even desirable to feed the same identical ration at all times, but the content of digestible nutrients has been kept as constant as possible throughout. The following is an accurate account of the various modifications in the rations:

HAY.—The hay fed was all grown locally, most of it on the station farm, and it has all been of the same general character, consisting for the most part of timothy with some redtop, bluegrass, and orchard grass. In all purchases of hay, a minimum of clover has been insisted on and none of the hay fed has contained more than a very small percentage of it. Some of the lots fed have not had even a stray stalk of clover. The average amount of calcium in these hays has been 0.47 per cent and of phosphorus 0.19 per cent. In order to impoverish the ration still further with respect to calcium and phosphorus, for the past year and a half a portion of the hay has been withheld and such mineral-poor materials as chopped oat straw, starch, and dried apple pomace have been successively substituted for it. The calcium and phosphorus content of these has been: Oat straw, 0.38 per cent Ca, 0.14 per cent P; starch, none; dried apple pomace, 0.11 per cent Ca, 0.11 per cent P.

Green soiling crops have been fed during the summer in place of a portion of the hay. During the first summer the experiment was in progress (1922) no departure was made from the long-established custom of feeding a maximum of 50 pounds of green feed per cow daily and of including in the succession of soiling crops such legumes as field peas and soy beans. Realizing that here might be a possible source of more calcium than would be desirable to feed under the conditions of the experiment, the amount fed was cut down last year (1923) to a maximum of 25 pounds daily, peas were eliminated from the rotation, and only a minimum of soy beans was fed. For a similar reason and as previously noted, the dry cows were not sent to pasture last summer (1923).

⁵ The writers in all their work have reduced their Ca and P figures to the elemental basis, not CaO and P₂O₅.

⁶ Results of this project are as yet unpublished.

GRAIN.—For the first 10 months of the experiment the grain ration consisted of—

	Pounds
Ground oats.....	39
Gluten meal.....	20
Corn meal.....	20
Wheat bran.....	20
Salt.....	1
Total.....	100

This combination contained about 15 per cent digestible protein, 55 per cent digestible carbohydrates and fat, 0.035 per cent calcium, and 0.40 per cent phosphorus.

In October, 1922, as already noted, chopped oat straw and starch were substituted for a portion of the hay. As a consequence of this change it became necessary, in order to balance the ration, to increase the protein content of the grain mixture. This was done by increasing the gluten meal to 30 pounds per hundred, and reducing the corn meal to 10 pounds. The resulting grain mixture contained about 18 per cent digestible protein, 52 per cent digestible carbohydrates and fat, 0.035 per cent calcium, and 0.42 per cent phosphorus.

The ration was again modified in September, 1923, dried apple pomace being substituted for the starch, the oat straw having been discontinued when the feeding of green fodder commenced in June. It occurred to the writers at this time (September, 1923) that possibly such a high percentage of gluten meal in the ration was not desirable, so the amount was reduced to 20 pounds, and in order to keep up the protein content 20 pounds of red-dog flour were substituted for the corn meal, so that the ration now being fed consists of:

	Pounds.
Ground oats.....	39 $\frac{1}{4}$
Gluten meal.....	20
Red-dog flour.....	20
Wheat bran.....	20
Salt.....	$\frac{3}{4}$
Total.....	100

This combination contains about 17 per cent digestible protein, 53 per cent digestible carbohydrates and fat, 0.06 per cent calcium, and 0.44 per cent phosphorus.

The foregoing variations in grain and roughage have not materially changed the general character of the ration, which, as already noted, has been formulated so as to be low in its content of calcium and phosphorus. Judged according to Kellner's (20, p. 618) standard for these elements, the rations have averaged throughout the experiment about 33 per cent deficient in calcium but have supplied a 62 per cent excess of phosphorus, thus showing that ordinary rations are much more likely to be lacking in calcium than in phosphorus, due to the relatively high percentages of phosphorus in all grains.

All the cows in the herd have received this ration, and, in addition, one-half of the herd, known as the "mineral" group, have received supplemental calcium and phosphorus in the form of a steamed bone meal, especially prepared for animal feeding. The bone meal has been added to each cow's daily grain ration at the time of weighing, being thoroughly mixed with the grain before feeding. The amount

for each cow, from 3 to 8 ounces daily, has varied with her weight and the amount of milk she gives, the aim being to supply approximately a 60 per cent excess of calcium over and above the theoretical requirements of Kellner's standard (20, p. 618). This excess is provided simply as a safeguard. Little is known of how completely such substances are assimilated, but it is safe to assume that they are by no means completely utilized, and just as there must be provided an excess of total organic nutrients in order to have a sufficiency of digestible organic nutrients, so there must be an excess of total mineral matter. The 60 per cent excess is an arbitrary figure.

Supplying this amount of calcium in the form of tricalic phosphate has also greatly increased the amount of phosphorus, so that the surplus of that element furnished to the "mineral" group has averaged around 160 per cent. This does not mean, however, that the ratio of acid to base in the ash of the total ration has been materially increased. The rations fed as just outlined have had a considerable excess of bases in their ash, and as the bone meal itself has an excess of base over acid, the addition of the mineral supplement, although it has relatively enhanced very much the excess of phosphorus over and above requirements, has intensified rather than minimized the basic nature of the ash.

ANALYTICAL PROCEDURE

Fodder and ash analyses of all materials fed have been made as the experiment progressed. Total solids and fat have been made monthly on the milk from every cow milking at the time, and in addition the total ash of all milks has been determined in 12 of the 30 months during which the experiment has been in progress, and over a period extending from June, 1922, to March, 1924. The calcium and phosphorus in the ash of the milks has been determined at nine different times and over a period extending from October, 1922, to March, 1924. A determination of the calcium and phosphorus in the drinking water supplied to the cows has also been made,

The methods of analysis have in all cases been those of the Association of Official Agricultural Chemists as described in the manual issued by that association (2).⁷

The foregoing is an accurate outline of the method of procedure in this "long-time feeding experiment with dairy cattle under conditions of practice." What are the results? After two and one-half years of continuous feeding to half the herd of a ration deficient in calcium and none too liberal in phosphorus, and to the other half the same ration plus sufficient supplemental mineral matter to provide a considerable surplus of both of these elements, what can be said either for or against the practice of supplying mineral matter in this form? The answer, so far as we have gone, is given in the next section and in the summary which follows it.

PRESENTATION AND DISCUSSION OF RESULTS

GENERAL CONDITION OF THE ANIMALS

An endeavor has been made to follow the condition of the animals closely, and observations have been recorded from month to month.

⁷ Acknowledgment is made here of the services of P. H. Smith and F. J. Kokoski of the feed laboratory and of H. D. Haskins and L. S. Walker of the fertilizer laboratory, who did practically all of the analytical work in connection with the project.

At no time has it been possible to note any marked difference in the aged cows. There have been times when, as a group, they have not been in the best of condition, but this has been true of the whole aged herd and not of any particular individual or group. Of the 14 aged cows with which the experiment was commenced, 9 are still in the herd, 4 of them being in the "mineral" group and 5 in the "non-mineral" group. (See Table I.)

TABLE I.—*History and present condition of the aged cows*

Cow No.	Age in years	Breed	Group	Number of calves		Present condition (May, 1924)
				Previous to commencement of experiment	Since commencement of experiment	
6.....	7	Grade Holstein.....	Nonmineral.....	1	2	Good.
8.....	12	do.....	do.....	^a 1	2	Fair.
9.....	9	do.....	Mineral.....	^b 1	^c 2	Good.
10.....	10	do.....	Nonmineral.....	5	2	Do.
11.....	10	do.....	Mineral.....	4	^d 2	Do.
12.....	7	do.....	do.....	1	3	Do.
16.....	11	Grade Jersey.....	Nonmineral.....	6	2	Do.
17.....	11	Purebred Jersey.....	Mineral.....	7	2	Fair.
19.....	7	Grade Jersey.....	Nonmineral.....	2	2	Good.

^a This cow was purchased when 7 years old. Her calf record previous to purchase in 1920 is unknown.

^b Purchased in 1921. Calf record previous to purchase unknown.

^c One of these was aborted at 7 months.

^d One of these was aborted at 5 months.

In so far as the eye can detect, none of these cows are undernourished or deprived of anything. As seen by their photographs (pls. 1 and 2), they are above the average in appearance, and even a keen observer would see nothing radically wrong with any of them.

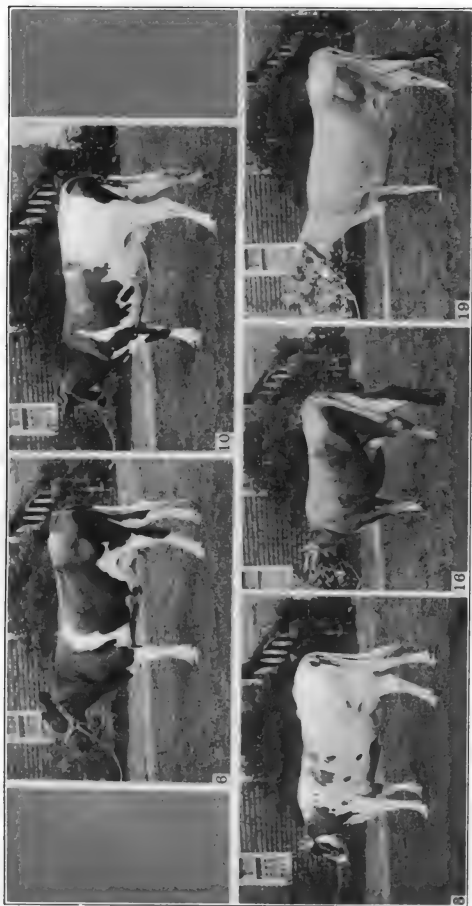
Another group of 6 individuals consists of 6 young cows which were either heifers or calves when the experiment commenced. (See Table II.)

TABLE II.—*History and present condition of the young cows*

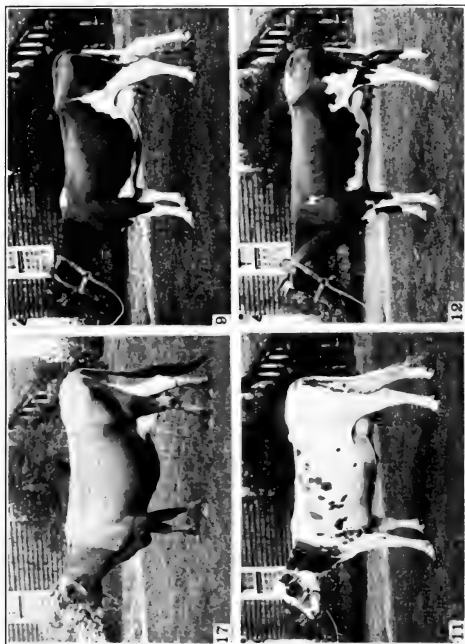
Cow No.	Age in years	Breed	Group	Number of calves		Present condition (May, 1924)
				Previous to commencement of experiment	Since commencement of experiment	
3.....	3	Grade Holstein.....	Mineral.....	None.	1	Fair.
5.....	3	do.....	Nonmineral.....	None.	1	Good.
15.....	5	do.....	do.....	^a None.	2	Do.
22.....	4	Grade Jersey.....	Mineral.....	None.	1	Poor.
23.....	4	do.....	Nonmineral.....	None.	2	Very poor
24.....	3	do.....	Mineral.....	None.	2	Poor.

^a All except No. 15 too young to breed; No. 15 had been bred but had not calved.

Here the situation is somewhat different. The last three cows in the list (pl. 3) are without a doubt suffering from lack of something in their ration, and that something is presumably calcium. It is perhaps



Aged cows, spring of 1924. Fed no mineral supplement



Aged cows, spring of 1924. Fed mineral supplement

significant that all three are Jerseys and that two of them have received the mineral supplement. The three Holsteins, on the contrary, have maintained themselves in a good state of nutrition, and it is rather paradoxical that the two that have not received any mineral supplement are in excellent condition, as good as any of the cows in the herd (pl. 3).

A third group consists of five heifers born since the commencement of the experiment. (See Table III.)

TABLE III.—*History and present condition of the heifers*

Heifer No.	Age in months	Breed	Group	Present condition (May, 1924)
40.....	21	Grade Holstein.....	Mineral.....	Good. ^a
42.....	19do.....	Nonmineral.....	Do.
43.....	19	Purebred Jersey.....	do.....	Excellent. ^b
57.....	9	Grade Holstein.....	do.....	Fair. ^b
59.....	8	Jersey-Ayrshire cross.....	do.....	Poor. ^b

^a Recently bred.

^b Unbred.

The last two of these are too young to pass judgment on, at least from this standpoint. As the progeny of cows in the experiment they will be discussed later on. The first three apparently have not as yet felt any bad effects of the low ash rations, nor has the mineral supplement added to No. 40's ration had any perceptible effect. She and her mate (No. 42) are, to all outward appearances, in identical condition (pl. 4).

The question will probably be raised as to why there are four heifers in the "nonmineral" group and only one in the "mineral" group. The procedure which, with a few exceptions, has been followed, has been to place the heifer calves in the same group as their dams, to see if any effects of the experiment might be cumulative. This past year, due to unfortunate circumstances in no way connected with the experiment, three heifer calves from cows in the "mineral" group have died, thus making an uneven division of the young animals. This fault, if not corrected during the present season by natural increase, will be adjusted by placing calves from "nonmineral" cows in the "mineral" group.

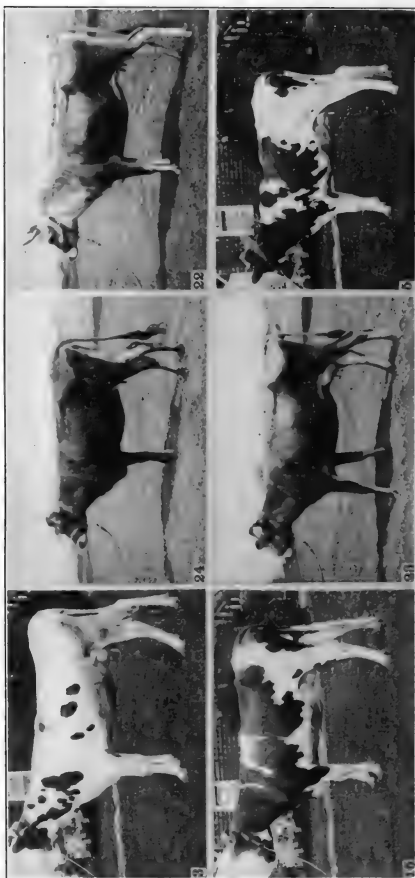
Summing up the evidence on all the individuals it is found, as far as general appearance and handling are concerned, that—

(1) The experimental treatment has had no prolonged ill effects on the aged cows, irrespective of breed.

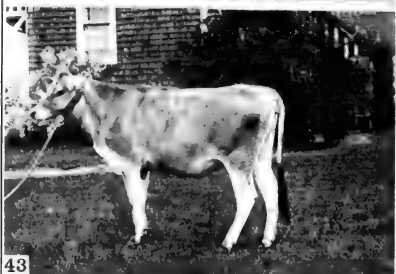
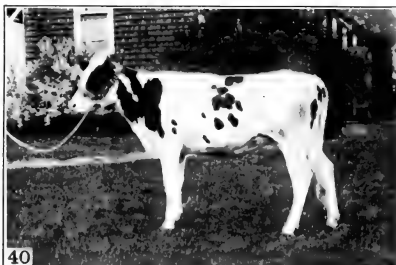
(2) The same is true so far of the heifers and of the young Holstein cows.

(3) The young Jersey cows, irrespective of the group they belong to, show conclusive evidence of impaired metabolism, in their general unthrifty appearance, thin flesh, and rough, harsh coats.

Not satisfied with their own judgment in the matter, the writers had the opinion of 10 impartial outsiders, all more or less experienced in the judging of cattle. A careful record of their decisions has been kept, and an analysis of this record shows that their decisions averaged 58 per cent correct, i. e., they had almost as many cows in the wrong group as in the right one. The mistakes were almost exactly divided between the two groups, 57.2 per cent of the mineral group



Young cows, spring of 1924. Nos. 3, 24, and 22 were fed mineral supplement. Nos. 13, 23, and 5 were not fed mineral supplement



Heifers, spring of 1921. No. 40 was fed mineral supplement. Nos. 42 and 43 did not receive mineral supplement

being rightly placed and 58.6 per cent of the nonmineral group. All agreed that, with two or three exceptions, there was no significant difference in the general appearance and handling of the cows.

BODY WEIGHT

For obvious reasons, only those cows which were mature at the beginning of the experiment can be considered under this heading. There are seven such individuals in the herd, and an endeavor has been made to have the weight for each of these at the beginning of the experiment and during the present season as comparable as possible. This has been done by noting the stage of lactation and gestation of each cow when the experiment started and by fixing a date during this present year at which time she was in approximately the same stage of gestation and lactation. An average of four separate weighings, one each on the first and second days of two consecutive months, has been taken in each instance. (See Table IV.)

TABLE IV.—*Net gain or loss in weight of the aged cows*

Cow No.	At beginning of experiment				Date this season when cow was at approximately the same stage of lactation and gestation	Average weight at that time	Gain or loss
	Age in years	Average weight in pounds	Stage in lactation in months	Stage in gestation in months			
Nonmineral group:							
8.....	9	1,215	4	(a)	June, 1924.....	1,235	+20
10.....	7	1,100	3	1	December, 1923.....	1,085	-15
16.....	8	910	11	6	February, 1923 ^b	920	+10
19.....	5	890	2	(a)	December, 1923.....	860	-30
Net gain or loss for the group.....							-15
Mineral group:							
9.....	7	1,230	3	1	October, 1923.....	1,205	-25
11.....	7	1,210	4	1	March, 1924.....	1,285	+75
17.....	9	870	2	(a)	December, 1923.....	850	-20
Net gain or loss for the group.....							+30

^a Not bred.

^b Can not get this cow with calf this year, so her weight a year ago is taken.

Because of the considerable variation in weights of cows, even from day to day, such small differences as noted above can not be considered of any significance.

MILK PRODUCTION

As noted elsewhere in this paper, Meigs (22) is of the opinion that cows fed rations deficient in mineral matter react to the deficiency by a marked decrease in milk flow from year to year.

The effect of the experiment in this respect has been noted by a careful study of the production records obtained by the writers. The results are presented in the accompanying charts, which show the average daily milk yield per cow by months, for the whole herd since January, 1920, and for the two groups since the commencement of the experiment in December, 1921.

It is quite evident (Chart I) that thus far the low-ash rations have had no appreciable effect on the milk yield of the herd as a whole. Also, it is equally evident (Charts II and III) that the mineral supplement has had no effect. The average daily milk yield of the cows in the mineral group has been slightly lower all through the

experiment than that of the cows in the nonmineral group. This can be ascribed in part to the slightly lower ratio of Holsteins to Jerseys in the mineral group, but computation shows that this is only a minor factor, and the lower yield must be considered as due chiefly to differences in the inherent ability of the individual cows to produce milk.

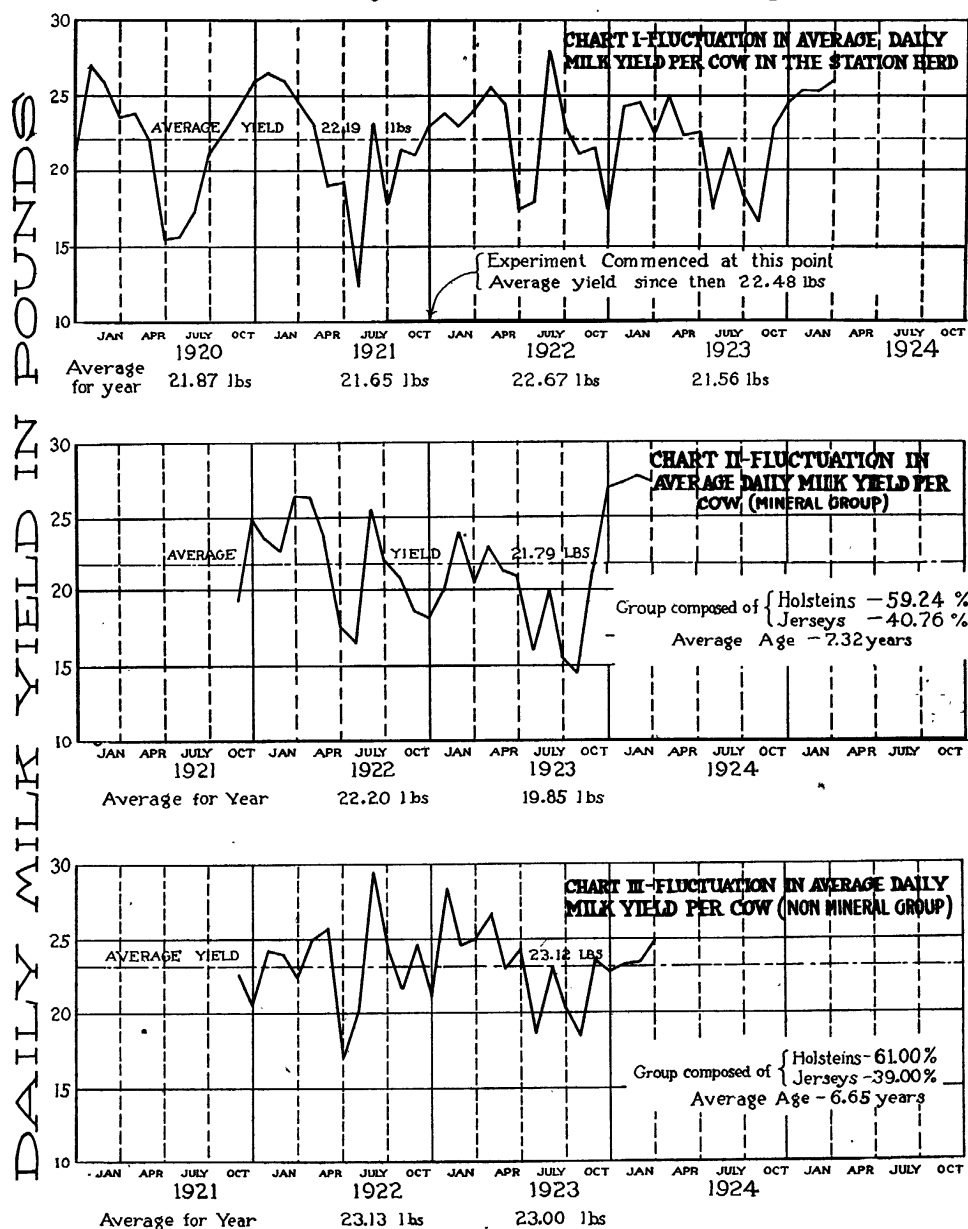


FIG. 1.—Charts showing fluctuation in the average daily milk yield per cow—for the station herd as a whole, for the group which was fed mineral supplement, and for the group which did not receive mineral supplement

COMPOSITION OF THE MILK

As already noted, five-day composite samples of each cow's milk have been taken during each month she has been in milk. Total solids and fat have been determined regularly on these samples. The total ash of the samples has been determined in 12 different months extending over a period from June, 1922, to March, 1924. The calcium and phosphorus of the ash have been determined in nine different months extending over a period from October, 1922, to March, 1924. The results are presented in Table V.

TABLE V.—Composition of milk

Period	Total solids	Fat	Total ash	Amount of ash in total solids	Calcium	Phos-phorus
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Preexperimental period, Jan., 1920, to Nov., 1921, whole herd	13.44	4.83	(*)	(*)	(*)	(*)
Experimental period, Dec., 1921, to Apr., 1924, whole herd	13.23	4.52	0.722	5.38	0.119	0.099
Mineral group	13.40	4.62	.721	5.28	.122	.099
Nonmineral group	13.06	4.42	.723	5.48	.117	.099

* Not determined.

The average content of total solids and fat has been slightly lower since the commencement of the experiment than it was during the two years previous, but this can be accounted for by the fact that since the experiment commenced the number of analyses of Jersey milk has been 6.94 per cent less than in the preexperimental period, while the analyses of Holstein milk have been 4.80 per cent greater. Due allowance having been made for this difference, it is seen that, taking the herd as a whole, the percentages of total solids and fat in the milk have not been materially affected by the conditions of the experiment.

The average for the mineral group is slightly higher than for the nonmineral group, but a similar reason to that given above accounts for the difference. While the number of Jersey milks tested was identical for each group, there were 14.6 per cent more Holstein milks from the nonmineral group than from the mineral group, which explains the somewhat lower fat and solid test of the former.

Respecting total ash and calcium and phosphorus, it is seen that with possibly one exception variations between the two groups are so slight as to be of no significance. The exception is calcium, which is slightly higher in the milks from the mineral group. It is possible that this may be due to the larger amount of calcium which the mineral group has received, but it is also possible that it may be due to inherited tendencies of the individual animals. As the figures are averages of 106 calcium determinations on milks from 18 different cows, it is unlikely that the difference is due to experimental or analytical error.

EFFECT ON REPRODUCTION

Under this heading have been observed:

(1) Recurrence of oestrus with respect to regularity and to the interval elapsing after calving before reappearance of oestrus. Regarding regularity, there are 19 individuals for which data are available. Scrutiny of their records reveals the following:

Regularity	Total	Mineral group	Non-mineral group
Good	13	6	7
Fair	5	3	2
Poor	1	1	0

In respect to the interval of time elapsing after calving before reappearance of oestrus, the average number of days throughout the course of the experiment has been:

	Days
Mineral group.....	39. 5
Nonmineral group.....	38. 6

These data show that both groups were quite similar in their behavior in these respects; in fairness, however, it must be stated that as the experiment has progressed the mineral group of cows has tended to come in heat sooner after calving, while the reverse has been true of the nonmineral group.

(2) Difficulties in getting cows bred. Considerable trouble has been experienced in getting the cows with calf, some of them having to be served four or five times before they would hold. The trouble has become somewhat more prevalent as the experiment has progressed, but has been quite evenly divided between the two groups, as shown by the accompany figures:

Group	Average number of times bred		
	1922	1923	1924
Mineral.....	1. 60	2. 25	2. 50
Nonmineral.....	1. 67	2. 67	2. 25

(3) Other abnormalities. There have been three cases of abortion and one premature birth in the herd since the commencement of the experiment. Two of the abortions were in the mineral group; the other one and the premature birth were in the nonmineral group. Two of the abortions are known to have been traumatic, and the others probably can not be attributed to the effect of the experiment.

There have been two cases of retained placenta, both in the nonmineral group; but one of them was in the case of a cow which had had the same trouble previous to commencement of the experiment.

(4) Condition of the calves at birth and subsequently. All calves dropped during the course of the experiment have either been raised to maturity or kept a sufficiently long time to enable an accurate opinion to be formed as to their constitutional vigor. Table VI summarizes the records.

TABLE VI.—Condition of calves

Group	Vigorous	Good	Fair	Delicate	Died at birth	Abor-tions
Mineral:						
1922.....	1	1		1		1
1923.....	2		2	1	1	1
1924.....		1	2			
Total.....	3	2	4	2	1	2
Nonmineral:						
1922.....	1	1	2	2		
1923.....	2	2	2	1	1	1
1924.....	1			2		
Total.....	4	3	4	5	1	1

Relatively, each group has produced about the same proportion of vigorous and good calves. The mineral group has had a somewhat higher proportion of fair calves, while the nonmineral group has had relatively almost twice as many delicate ones. The deaths at birth and the abortions probably have no bearing on the experiment, but are presented in order that the statistics may be complete. It is perhaps significant that there have been a relatively large number of delicate calves from the nonmineral group. The weakness of constitution has manifested itself for the most part in a very delicate and sensitive digestive system. All of these delicate calves had to be pampered along, not even making satisfactory progress on a whole milk-skim milk diet, and in the end every one of them died or had to be killed. Post-mortem examination revealed in every instance more or less inflammation of the digestive tract, amounting in at least one case to a chronic gastrointestinal catarrh.

It would be unwise, however, to attach too much weight to this evidence, for other factors, such as the individuality and breed of the dams, has no doubt had considerable to do with the condition of the calves. All the vigorous and good calves have been from Holstein cows. All the fair and delicate ones, with four exceptions, have been from Jersey cows. Three of the four exceptions were heifer's calves, and were not sired by a Holstein bull.

SUMMARY

The object of this experiment has been to test the efficacy of adding supplemental inorganic salts of calcium to the rations of dairy cows.

The whole station herd has been fed for two and a half years on a ration decidedly low in calcium, too low, from the standpoint of our present knowledge, for the needs of the animals. One-half of the herd has had the deficiency made good theoretically by supplementing the ration with calcium phosphate in the form of special steamed bone meal.

The following are the outstanding facts revealed by the investigation thus far:

(1) Irrespective of the group to which they belong, the experimental procedure has had no prolonged ill effects on the general condition of the aged cows, of the young Holstein cows, or of the heifers, but has apparently seriously disturbed the metabolism of the young Jersey cows.

(2) Changes in body weight of the aged cows have been insignificant. Obviously, the young cows and heifers can not be considered under this heading.

(3) Milk production has not been significantly affected either by the low-calcium ration or by the mineral supplement, except in the case of the young Jersey cows. They have not milked as freely or as persistently as it was expected they would. Too much stress, however, should not be placed upon this latter statement, because of the small number of cows studied.

(4) With the possible exception of calcium, the composition of the milk has not been appreciably effected.

(5) The reproductive function has been more seriously disturbed than any other, considerable difficulty being experienced in getting

the cows with calf. This difficulty has increased as the experiment has progressed, but has been about equally divided between the two groups.

(6) Each group has produced about the same proportion of strong, healthy calves. The nonmineral group has had much the higher proportion of delicate calves, but this is due in part to breed characteristics and should not be taken too seriously.

(7) It should be borne in mind that this summary is a report of progress and should not be considered as final.

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THE WATER-SOLUBLE VITAMIN CONTENT OF THE VELVET BEAN¹

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INTRODUCTION

Brief studies of the water-soluble vitamin content of the velvet bean have been reported. Data collected at the Agricultural Experiment Station of the Alabama Polytechnic Institute in 1920-21 by Miller,² Burleson, and Templeton show that velvet beans or alcoholic extracts of velvet beans improved the condition of polyneuritic pigeons.

Salmon³ observed that polyneuritic pigeons were improved by the administration of ground velvet beans, and also that the onset of polyneuritis was delayed by furnishing an aqueous extract of velvet beans as the sole source of water to pigeons receiving a diet of polished rice.

Sure and Read⁴ report that when rats received 60 to 80 per cent of autoclaved velvet beans, or 40 per cent of the raw beans, as the source of vitamin B, little growth resulted. These investigators concluded that the velvet bean is very low in vitamin B.

These results led the writers to undertake a more complete investigation in which velvet beans from the same source would be tested on both pigeons and rats.

CURATIVE EXPERIMENTS WITH PIGEONS

A number of pigeons that had developed pronounced symptoms of polyneuritis on a diet of polished rice were restored to an apparently normal condition by 1 to 2 grams of finely ground velvet beans.⁵ One pigeon (No. 19) that was having spasms was given 1 gram of velvet beans. The bird appeared to be normal the next day, and no further symptoms of polyneuritis appeared for five days after the 1 gram of beans was administered.

Other pigeons were cured by alcoholic and acetic-acid extracts of velvet beans. Several of the polyneuritic pigeons that received such extracts were not cured, however. In most cases considerable improvement in the condition of the bird was noted, and in a few cases birds that had apparently been cured died a few days later.

PREVENTIVE EXPERIMENTS WITH PIGEONS

The preventive tests were begun in July, 1922, with velvet beans from the 1921 crop. The beans or extracts of the beans were fed by hand. Polished rice was kept before the birds at all times, except in the cases where it was fed by hand (noted in Table I).

¹ Received for publication Jan. 26, 1925; issued December, 1925. Published with the permission of the director of the Alabama Experiment Station.

² MILLER, E. R. DO VELVET BEANS CONTAIN VITAMIN B? (Abstract) *Science* 56: 25. 1922.

³ SALMON, W. D. THE EFFECT OF FEEDING VELVET BEANS TO PIGEONS. *Science* 56: 368. 1922.

⁴ SURE, B., and READ, J. W. BIOLOGICAL ANALYSIS OF THE SEED OF THE GEORGIA VELVET BEAN, *STILOBIUM DEERINGIANUM*. *Jour. Agr. Research* 22: 5-15, illus. 1921.

⁵ Shelled velvet beans, Early Speckled variety, were used in all tests reported in this paper.

TABLE I.—Record of pigeons by periods

	Length of period	Initial weight	Gain (+) or loss (—)	Average daily feed		
				Rice	Beans	Extract
	Days	Grams	Grams	Grams	Grams	Grams
Pigeon No. 8:						
Period A.....	41	299	—86	^a 11.4	-----	-----
Period B.....	49	213	+28	17.3	-----	^b 0.50
Period C.....	21	241	—18	10.0	-----	^c 0.25
Period D.....	35	223	+9	^a 12.1	5.0	-----
Period E.....	42	232	+38	^a 16.3	2.0	-----
Pigeon No. 1:						
Period A.....	7	320	—30	(?)	-----	-----
Period B.....	63	290	—17	16.3	-----	^d 0.38
Period C.....	70	273	+7	13.5	2.0	-----
Period D.....	14	280	—43	6.0	^e 2.0	-----
Period E.....	77	237	+5	12.0	1.0	-----
Pigeon No. 14:						
Period A.....	70	278	—50	16.5	-----	^f 0.67
Period B.....	70	228	+22	19.0	2.0	-----
Period C.....	21	250	—80	15.7	^e 2.0	-----
Pigeon No. 13:						
Period A.....	77	268	—13	^a 12.3	4.67	-----
Period B.....	28	255	+8	^a 16.5	1.80	-----
Pigeon No. 17.....	17	323	—130	^a 11.4	^e 4.0	-----
Pigeon No. 3:						
Period A.....	19	304	—74	19.4	-----	-----
Period B.....	7	230	—23	^a 17.0	-----	-----
Pigeon No. 4:						
Period A.....	19	301	—73	21.0	-----	-----
Period B.....	17	228	—9	^a 15.5	-----	-----
Pigeon No. 7.....	5	266	—87	-----	9.0	-----
Pigeon No. 11.....	9	264	—53	-----	11.1	-----

^a Polished rice hand fed.^b First percolate from 8 grams beans.^c Flask extraction from 2.77 grams beans.^d Second percolate from 4 grams beans.^e Velvet beans extracted by alcohol.^f Second percolate from 3.2 grams beans.

Table I and Figure 1 show a summary of the results. When alcoholic extracts of velvet beans were given to pigeons that were declining in weight on a diet of polished rice, the decline was checked and in some cases gains in weight occurred (periods B and C of No. 8, A of No. 14, and B of No. 1). Pigeon No. 8 developed polyneuritis on a diet of polished rice in period A. A daily dose of alcoholic extract ^a representing 8 grams of velvet beans was then given throughout period B. The bird was restored to an apparently normal condition and made decided gains in this period. The bird was then changed to a different alcoholic extract ⁷ in daily doses representing 2.77 grams of beans. The bird received this extract for only 21 days (period C). There was considerable loss of weight, but the pigeon was apparently in good condition at the close of the period.

That the extract given No. 8 in period B did not carry all the protective substance contained in the beans is shown by period A of No. 14 and period B of No. 1.

After the percolate had been removed for the preparation of the extract given No. 8 in period B, the percolation was continued and an extract prepared from the second fraction. Pigeons Nos. 1 and 14 received this second fraction extract representing 4 grams and 3.2 grams of velvet beans per day, respectively, for 70 days. Both birds declined in weight considerably, but did not show any symptoms of polyneuritis. The longest-surviving check birds (Nos. 3 and 4) lived only 26 and 36 days, respectively, after they were started on polished rice.

⁶ This extract was prepared by percolating ground velvet beans with cold 90 to 95 per cent alcohol, and evaporating the percolate on dextrin.

⁷ This extract was prepared by extracting velvet beans on boiling water bath with four fractions of 90 to 95 per cent alcohol, each fraction being heated two hours.

In these tests raw velvet beans were more efficient than the extracts. Daily doses of 2 grams of the raw beans gave complete protection and enabled birds weighing 220 to 270 grams to maintain their weight or make slight gains through periods of 40 to 70 days (periods D and E of No. 8, A and B of No. 13, C of No. 1, and B of No. 14).

Velvet beans that had been extracted with alcohol in Soxhlet for 48 hours did not have any protective action (pigeon No. 17, and periods C of No. 14 and D of No. 1). Pigeon No. 17 received 4 grams of the extracted material per day, and pigeons Nos. 1 and 14

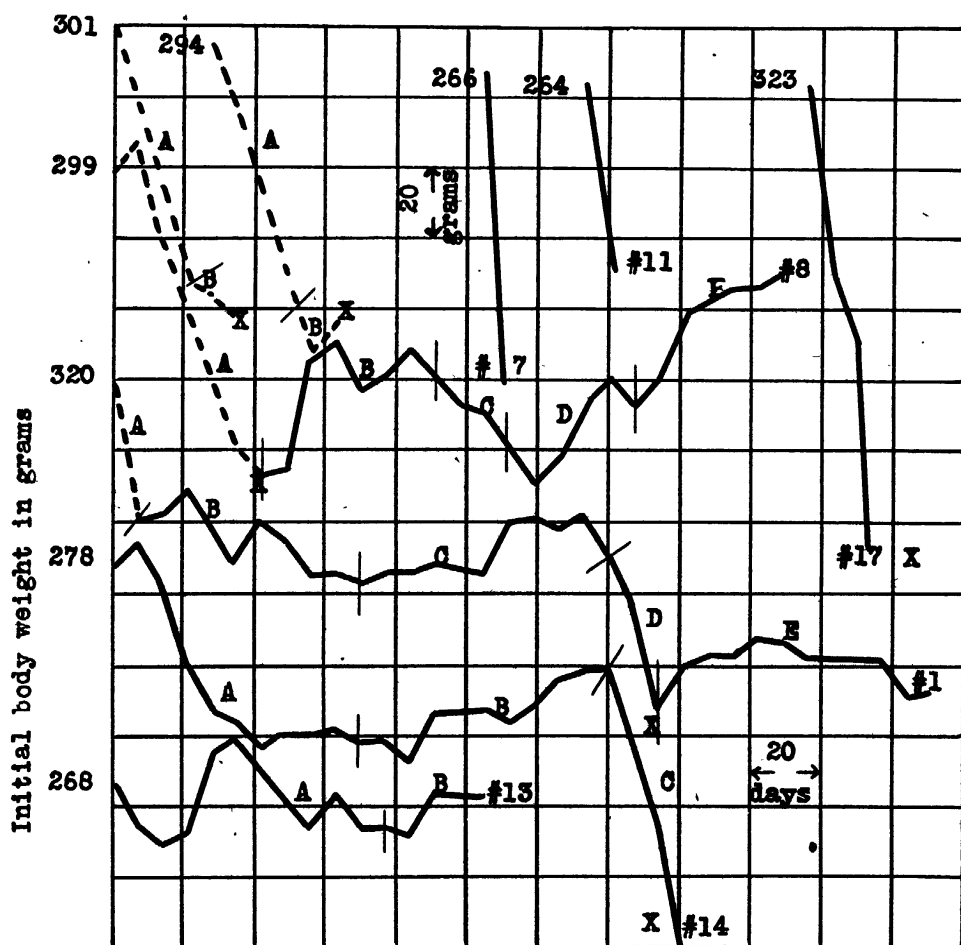


FIG. 1.—Weight curves of pigeons: Effect of feeding velvet beans. Letters refer to the periods in Table I. Broken lines indicate periods in which polished rice alone was fed. X indicates onset of polyneuritis.

received 2 grams each per day. All three birds lost weight rapidly and came down with polyneuritis in 14 to 21 days.

After pigeon No. 1 was unable to stand, it was given 2 grams of raw velvet beans, and it appeared to be almost normal the next day. This bird was again given 2 grams of raw beans on the second day, and then 1 gram per day for 77 days (period E). No symptoms of polyneuritis appeared during this period.

Pigeons Nos. 7 and 11 received raw velvet beans alone, hand fed (no rice). They lost weight rapidly and died on the fifth and ninth days, respectively. The harmful effect of a diet of velvet beans alone has previously been noted.⁸

⁸ SALMON, W. D. THE EFFECT OF FEEDING VELVET BEANS TO PIGEONS. *Science* 56: 368. 1922.

GROWTH EXPERIMENTS ON RATS

The usual laboratory routine for vitamin B tests was followed in these experiments. Young white rats were caged individually on screens which prevented access to excreta. The rats were fed once each day and weighed once a week. A record of feed consumption was kept.

The following basal diet was fed until the rats were stationary or declining in weight:

	Per cent
Casein ⁹	18.0
Salts (McCollum's No. 185).....	3.7
Agar.....	2.0
Rice powder ¹⁰	71.3
Butter fat ¹¹	5.0

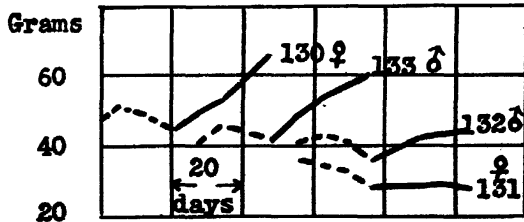


FIG. 2.—Weight curves of rats. Basal diet, plus 10 per cent raw velvet beans. Broken lines in charts 2 to 10 represent periods in which the basal vitamin B free diet alone was used

The velvet beans or extracts were then added to the basal diet, replacing an equal amount of rice powder. When yeast was added, an amount of dried yeast¹² equal to 5 per cent of the daily allowance of feed was placed on top of the other feed in the feeder.

The addition of 10 per cent of raw velvet beans to the basal diet checked the decline of rats and enabled them to make very slight gains (fig. 2).

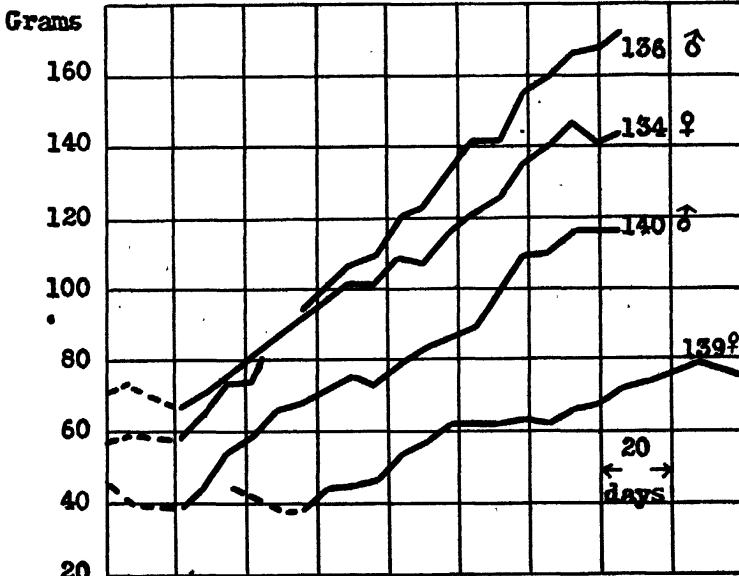


FIG. 3.—Weight curves of rats. Basal diet, plus 20 per cent raw velvet beans

When 20 per cent of raw beans was added to the basal diet the rats grew at approximately one-third of the normal rate (fig. 3).

When 5 per cent of dried yeast was added to the above diet normal growth occurred (fig. 4). The females were bred, and then littered

⁹ Washed one week in 0.2 per cent acetic acid.

¹⁰ Heated in electric oven five hours at 120° C.

¹¹ Thoroughly washed and centrifuged.

¹² Dried yeast (Harris).

young that were nearly normal in weight but raised none of them to weaning. After the addition of 2.5 c. c. of orange juice per day to the diet, female No. 71 raised part of the next litter, but the young did

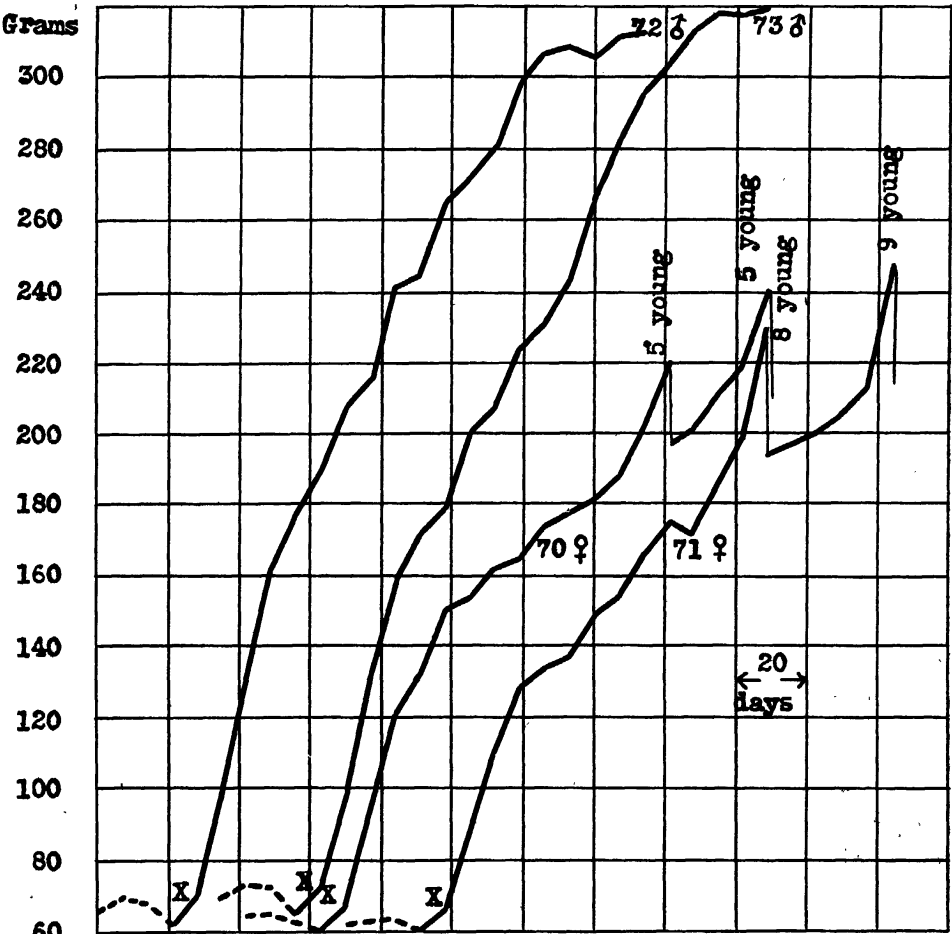


FIG. 4.—Weight curves of rats. Basal diet, plus 20 per cent raw velvet beans. At point X, 5 per cent of dried yeast was added.

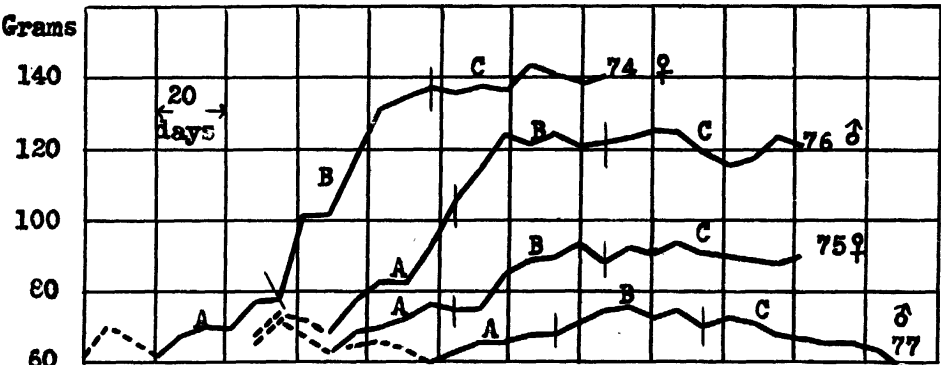


FIG. 5.—Weight curves of rats. Basal diet, plus 20 per cent cooked velvet beans. Periods A and C; 20 per cent raw velvet beans Period B

not make normal growth. After the addition of 10 c. c. of whole milk per day, female No. 70 raised the second litter successfully, and the young grew to maturity at the normal rate on the diet of the mother.

Velvet beans cooked on boiling-water bath for one and one-half hours were no more efficient as a source of vitamin B than raw velvet beans, when both were fed at a concentration of 20 per cent (fig. 5).

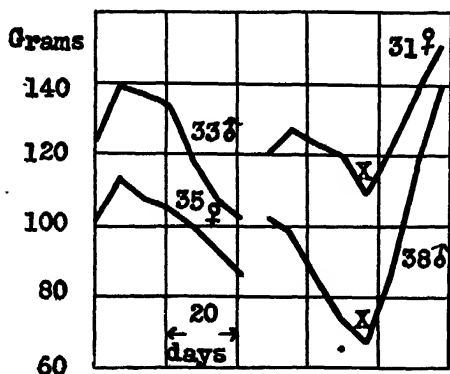


FIG. 6.—Weight curves of rats. Basal diet, plus 20 per cent velvet beans previously extracted with alcohol. At point X, 5 per cent dried yeast was added

effect of the larger percentage of beans. The addition of 5 per cent of yeast to the diet that contained 50 per cent of raw beans resulted

in slight improvement in the growth rate (fig. 8), but did not produce as satisfactory growth as the addition of a like amount of yeast to the diet that contained only 20 per cent of raw beans. There was considerable diarrhea among the rats that received the diet containing 50 per cent of raw beans.

The results indicate that the harmful effect of the beans was the most important limiting factor in this diet, although the 50 per cent

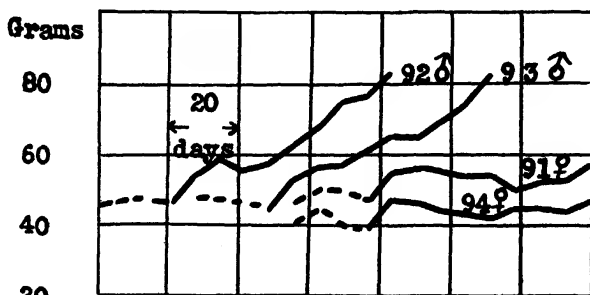


FIG. 7.—Weight curves of rats. Basal diet, plus 50 per cent raw velvet beans

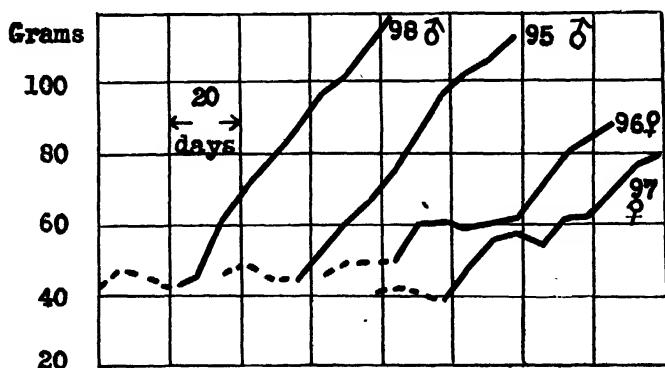


FIG. 8.—Weight curves of rats. Basal diet, plus 50 per cent raw velvet beans, plus 5 per cent yeast

of raw beans apparently did not furnish the optimum supply of vitamin B.

A comparison of Figures 8 and 9 shows that cooking the beans on boiling-water bath for one and one-half hours lessened the harmful effect. The feces of the group receiving 50 per cent of cooked beans (with yeast) were normal,

and the rats remained clean and plump, in marked contrast to the soiled, unthrifty appearance of those that received 50 per cent of raw beans. Growth was not as satisfactory, however, on 50 per cent of cooked beans (yeast added) as on 20 per cent of raw beans (yeast added).

Autoclaving the beans for two hours at 15 pounds pressure lessened their harmful effect but did not give better results than cooking on the water bath.

Various alcoholic and acetic-acid extracts were made in an effort to obtain the growth factor free from the harmful substance. These extracts were added to the basal diet to represent varying percentages of beans, but in all cases the extracts were less potent than the same percentage of raw beans.

SUMMARY

Polyneuritic pigeons were restored to apparently normal health by raw velvet beans and by alcoholic and acetic-acid extracts of velvet beans. Pigeons weighing 220 to 270 grams and receiving a diet of polished rice were protected against the onset of polyneuritis by 2 grams of raw velvet beans per day. This amount represented 9.5 to 13 per cent of the total daily feed. Velvet beans that had been thoroughly extracted with alcohol had no protective action when fed in doses of 2 to 4 grams per day.

In one case, 1 gram of raw velvet beans per day prevented the onset of polyneuritis during a period of 77 days' feeding on polished rice.

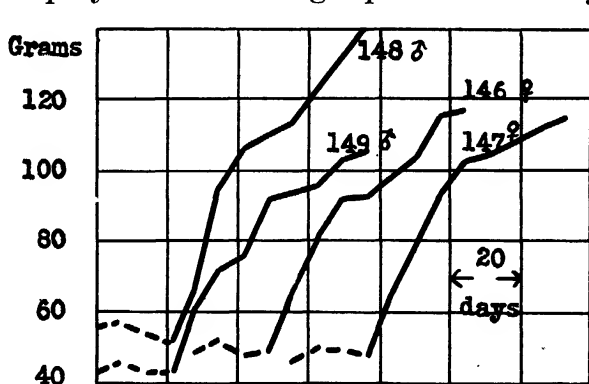


FIG. 10.—Weight curves of rats. Basal diet, plus 50 per cent autoclaved velvet beans, plus 5 per cent yeast

than on diets that contained either 10 per cent or 50 per cent of beans.

The addition of 5 per cent of yeast to diets carrying 20 per cent or 50 per cent of raw beans caused a marked increase in the rate of growth. The effect was more pronounced, however, in the case of diets carrying the 20 per cent concentration of beans.

Cooking velvet beans one and one-half hours on boiling water bath lessened but did not entirely overcome their harmful effect on rats.

Autoclaving the beans for two hours at 15 pounds pressure was no more beneficial than cooking them as described above.

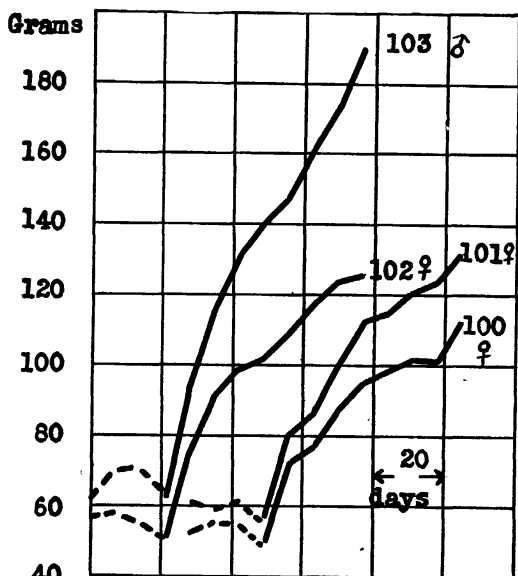


FIG. 9.—Weight curves of rats. Basal diet, plus 50 per cent cooked velvet beans, plus 5 per cent yeast

Although small amounts of velvet beans had a protective action, large amounts were harmful. This was shown by the death of two pigeons in five and nine days, respectively, when they were fed beans alone.

When raw velvet beans were used as the sole source of vitamin B, rats grew more rapidly on diets that contained 20 per cent of beans

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STUDIES ON *OPHIOBOLUS GRAMINIS* SACC. AND THE TAKE-ALL DISEASE OF WHEAT¹By RAY J. DAVIS²*Formerly Field Assistant, Office of Cereal Investigations, Bureau of Plant Industry,
United States Department of Agriculture*

INTRODUCTION

This paper deals with laboratory, greenhouse, and limited outdoor studies on the take-all disease of wheat and the causal fungus. Since this disease does not occur in the region where these studies were made, it was not practicable to make extensive field studies.

MATERIALS AND TECHNIQUE

ORGANISM

Three single-spore strains of *Ophiobolus graminis* Sacc., isolated from diseased plants growing in widely different regions, were supplied the writer by H. H. McKinney, of the United States Department of Agriculture, and these have been used throughout the investigations. One of these strains came originally from R. S. Kirby, of Cornell University, who isolated it from a wheat plant affected by take-all in New York State. This strain is referred to throughout this paper as the "New York strain." The other two strains were isolated by Hurley Fellows, of the United States Department of Agriculture, from take-all diseased wheat plants growing near Hillsboro, Oreg., and Fayetteville, Ark., respectively, and are referred to throughout the paper as the "Oregon strain" and the "Arkansas strain."

MEDIA

Preliminary experimentation dealing with the growth and sporulation of the organism on different media led to the adoption of the following three media for the greater part of the work: (1) potato-dextrose agar, (2) string-bean agar, and (3) cooked kernels of barley and oats (equal portions).

In the physiological experiments potato-dextrose agar was used as the principal substratum. Nutrient broth and Czapek's full nutrient solution also were employed in the studies on the growth of the fungus in relation to hydrogen-ion concentration.

Potato-dextrose agar was prepared, according to the usual laboratory method, as follows: Two hundred grams of peeled potatoes were cut into small pieces, placed in 1,000 c. c. distilled water, and steamed

¹ Received for publication Feb. 2, 1925; issued January, 1925. These studies were carried on cooperatively by the Office of Cereal Investigations, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, Madison, Wis.

² The writer wishes to express his appreciation to L. R. Jones, James G. Dickson, and H. H. McKinney for their many valuable suggestions during the progress of these studies, and to Mr. McKinney and A. G. Johnson for assistance in revising the manuscript.

in an Arnold sterilizer for approximately an hour. The liquid was then strained off and the original volume of liquid restored by addition of distilled water. Agar 20 gms. and dextrose 20 gms. were then added and steamed a short time, and filtered while hot through absorbent cotton. Equal quantities of the medium were then placed in flasks and sterilized in an autoclave for 30 minutes at 10 pounds pressure. This medium was chosen as a standard and all stock cultures were grown on it.

The nutrient broth was made by adding three grams of Liebig's beef extract and 10 gms. of peptone to 1,000 c. c. of distilled water; the mixture was warmed and stirred until the peptone was dissolved, then filtered, placed in flasks, and sterilized in an autoclave for 30 minutes at 10 pounds pressure.

Czapek's full nutrient solution was made according to the following formula:

Magnesium sulphate (MgSO_4)	gm.	0.5
Potassium dihydrogen phosphate (KH_2PO_4)	gm.	1.0
Potassium chloride (KCl)	gm.	.5
Sodium nitrate (NaNO_3)	gm.	2.0
Ferrous sulphate (FeSO_4)	gm.	.01
Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	gm.	30.0
Distilled water to make	c. c.	1,000

Equal quantities were placed in flasks and sterilized for 30 minutes at 10 pounds pressure. The methods employed in adjusting the various media to different hydrogen-ion concentrations are described later under the heading of the influence of hydrogen-ion concentration on growth of the fungus.

String-bean agar, which proved to be most satisfactory for perithecial production, was prepared in the same manner as potato-dextrose agar, except that string beans were substituted for potatoes and the dextrose was omitted.

A medium made from equal parts of oats and barley kernels proved to be favorable for increasing the fungus for purposes of inoculating soil. This medium enabled the fungus to make a luxuriant growth, and very little if any deleterious effect on the wheat plants resulted when the uninoculated medium was used in the soil in the controls. The grain mixture for this medium was first soaked over night in cold water and then boiled in an excess of water over a free flame for about an hour. Then 500 and 1,000 c. c. of the cooked kernels were placed in Erlenmeyer flasks of 1 to 2 liters capacity, respectively. Enough of the decoction water was added to each flask to keep the kernels moist, but not too wet, throughout the period the fungus was growing. The flasks were plugged with cotton and sterilized 45 minutes at 15 pounds pressure.

Following the inoculation, and after the fungus had thoroughly grown through the mass of kernels, the contents of the flasks were used to inoculate experimental soil. An equal quantity of inoculum was added to and thoroughly mixed with the upper soil in each container. Likewise in controls an equal quantity of the uninoculated medium was added. Immediately following this treatment wheat was sown in the inoculated and uninoculated soil.

Other media, especially cooked wheat kernels, produced a somewhat better growth of mycelium, but it was found that these media were toxic to wheat plants.

A modified Crone's nutrient agar, made according to the following formula, was used as the medium in which to grow wheat plants under aseptic conditions:

Potassium nitrate (KNO_3)	-----gm--	1. 0
Magnesium sulphate (MgSO_4)	-----gm--	0. 25
Calcium sulphate (CaSO_4)	-----gm--	. 25
Calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$)	-----gm--	. 25
Iron sulphate (FeSO_4)	-----gm--	. 25
Distilled water to make	-----c. c.--	1, 000. 00
Agar	-----gms--	10. 00

SEEDS

Three varieties of wheat were employed in these experiments, namely, Goldcoin (Junior No. 6), California Club, and Marquis. The first named was used in the greater part of the work. The kernels were, in all cases, sterilized 10 minutes in 1-1000 HgCl_2 , washed thoroughly in running water, dried at room temperature, and planted $1\frac{1}{2}$ inches deep at the rate of 1 gram per linear foot.

SOIL

The soil used in all of the experiments was a silt loam, obtained at the base of a hill covered with trees and shrubs, and was, therefore, reasonably free from general field-crop soil-infesting organisms. It was black, very rich in organic matter, and had a hydrogen-ion concentration of about 6.6 P_H after sterilization for 2 hours at 15 pounds pressure.

TAXONOMY OF THE FUNGUS

The taxonomy of the take-all fungus is discussed by McKinney (10)³ and is not taken up in detail here. The parasite was described as *Ophiobolus graminis* by Saccardo (14, p. 349) and this name has been generally accepted. Recently Fitzpatrick, Thomas, and Kirby (5) concluded that this name is invalid. However, as pointed out by McKinney (10), there seems to be justification for continuing to use Saccardo's name and therefore the writer is following this older and generally accepted usage.

MORPHOLOGY OF THE FUNGUS

Throughout the course of these studies numerous mature perithecia were produced by the New York strain of the fungus when grown in pure culture. Perithecia were also produced by this strain on wheat plants grown under controlled experimental conditions. These fruiting bodies varied greatly in size and shape, but the most of them were typical for *Ophiobolus graminis*. They were always black in color, had loose strands of mycelium about them, and averaged about 300 microns in diameter; most of them had beaks, which were usually curved. However, the beaks were extremely variable in length and shape.

Asci were produced in abundance in pure culture. They came out of the perithecium singly, and as they left the ostiole they were shot out for a short distance. The ascus wall apparently dissolved, usually beginning at one end, when it came in contact with the water,

³ Reference is made by number (italic) to "Literature cited," p. 825.

and in less than an hour the spores began to separate. No other method of spore liberation was noted.

Spore germination was studied, but only one method was found to occur. In from six hours to two or three days, germ tubes developed and branched and rebranched, and soon formed a network of hyphae. The end cells of a spore germinated most frequently, but any cell

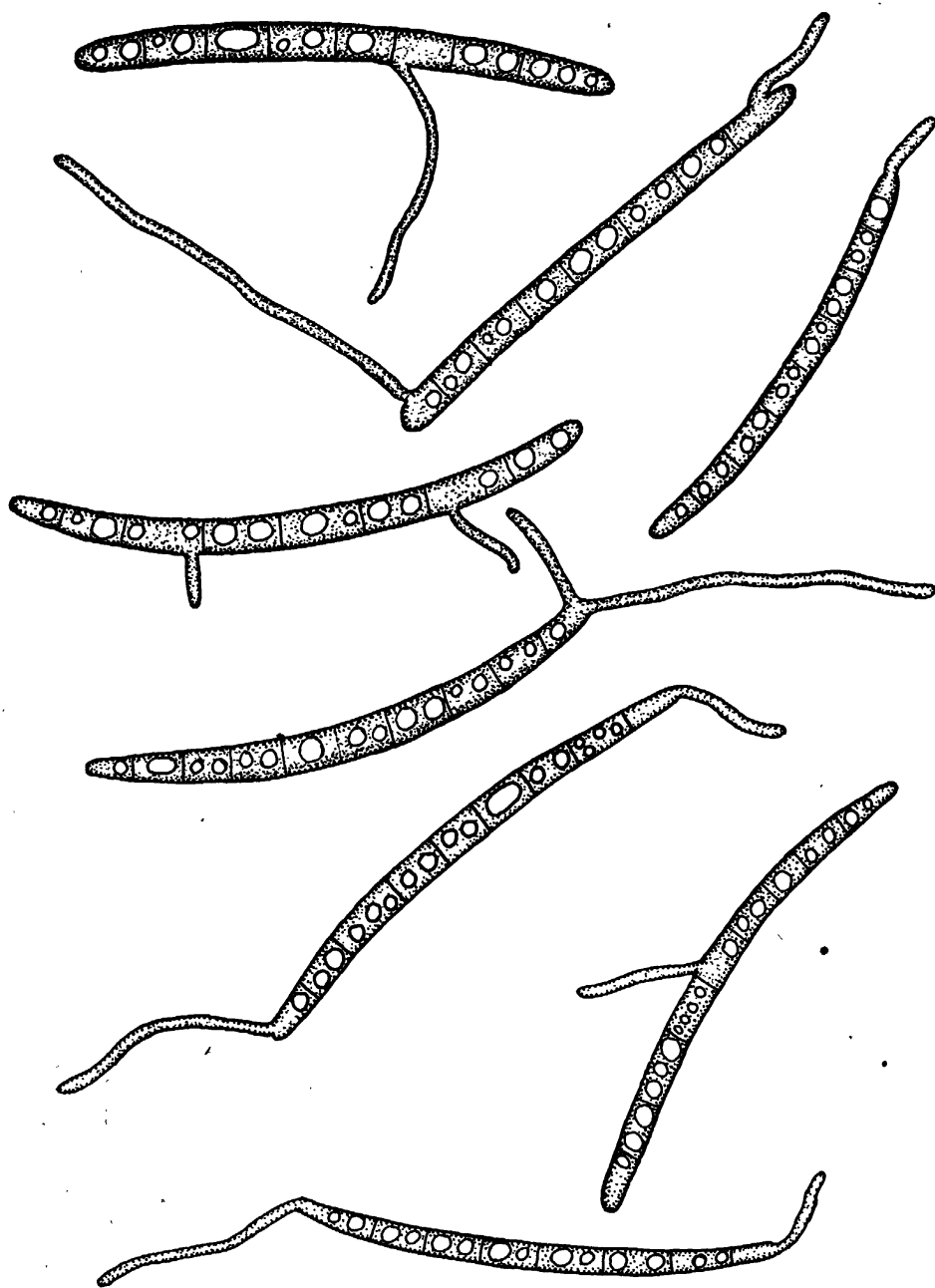
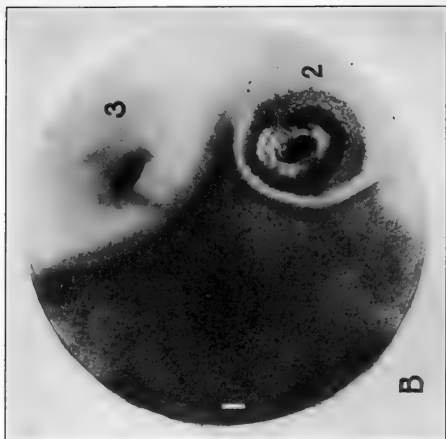
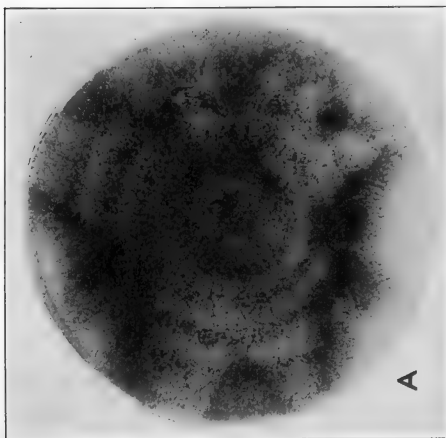


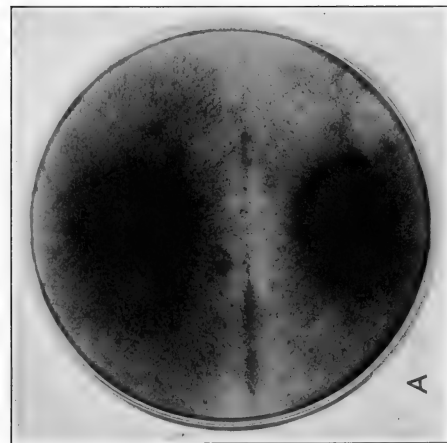
FIG. 1.—Germinating ascospores of *Ophiobolus graminis*, showing germ tubes coming from various cells of the spore. Drawn with aid of camera lucida. $\times 1,000$

of the five to nine present was capable of germination (fig. 1), and as many as three were seen to germinate in a single spore. Hori (6) claims that only the end cells of the ascospore germinate, but the writer repeatedly noted that the other cells produced germ tubes.

Mangin (12) claims that on germination the spores form a promycelium which bears sickle-shaped sporidia, and that these give rise to germ tubes which enter the host plants through the root hairs.



A.—A single spore culture of the New York strain of *Ophiobolus graminis*. Mature perithecia can be seen in concentric circles about the point of inoculation.
B.—A Petri-dish culture of *Ophiobolus graminis*, inoculated at three points, with as many different strains, as follows: (1) New York, single-spore, strain; (2) Oregon strain; (3) Arkansas strain. [Note the perithecia in the colony of the New York strain, which examinations revealed to be mature]



A.—Culture inoculated at two points with the same single-spore strain, and incubated in diffused light. Mature perithecia were produced at or near the line of contact of the two colonies

B.—Same as A, except three inoculations were made and the culture grown in darkness. (Note the absence of perithecia and the characteristic type of hyphae massed in compact strands. These strands are similar to those which occur in the mycelial plates under the leaf sheaths of infected wheat plants)

However, Delacroix (3) made further observation in an attempt to verify Mangin's work, but failed to find promycelia and sporidia. He suggested that Mangin probably had another species of *Ophiobolus*.

In the course of the present studies, no spore forms other than ascospores have been found. Paraphyses were present in the perithecia during ascus formation, but as the spores matured they disappeared. Perithecia in which the asci were well developed, but in which no spores were differentiated, contained paraphyses. The paraphyses were hyaline, three to four celled, slightly broader at the base than at the tip, and rather irregular in shape. When the ascospores were formed, the paraphyses disappeared. Apparently they were absorbed to furnish food for the developing spores, as no trace of them could be seen in any fully matured perithecium.

The mycelium was found to be extremely variable both in general appearance and diameter of hyphae, but this was probably due in part to age and conditions of growth. In rapidly growing cultures a fine, hyaline mycelium with granular protoplasm and few or no cross-walls was very abundant. As the hyphae became older, they increased in diameter, the walls became brown in color, and numerous

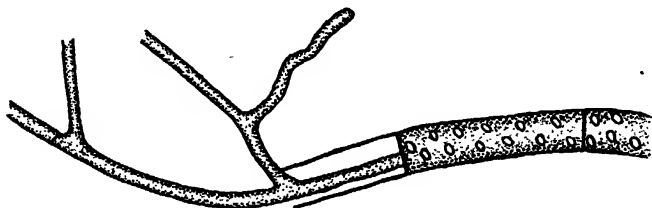


FIG. 2.—Fine hyaline mycelium developed at a broken end of a coarse brown hypha of *Ophiobolus graminis*. Drawn with aid of a camera lucida. $\times 1,000$

septa were formed, the protoplasm lost its granular appearance, and oil droplets appeared in the cells. Furthermore, an injury to a coarse hypha often would induce the formation of a new hypha at the point of injury. This new mycelium invariably had a much smaller diameter than the old, as shown in Figure 2. With ordinary staining methods the fine mycelium readily took up and retained the stain, while the coarse type did not hold the stain well. Very often the mature hyphae adhered closely together and formed strands. As many as 6 hyphae have been seen united in this manner, and when attempts were made to separate them the whole strand often broke crosswise.

As stated previously, pure cultures of the New York strain of the fungus formed numerous mature perithecia when grown on artificial media. However, the Oregon and Arkansas strains of the parasite never produced mature fruiting bodies when handled in the same manner as the New York strain. In some cases perithecium-like bodies were produced, but these were never found to contain asci or ascospores. When the Oregon and Arkansas strains were planted on opposite sides of a Petri dish containing solid medium, they seldom grew together. When they did, however, the hyphae were much darker in color along the line where the two mycelia met than elsewhere, but in no case did mature fruiting bodies develop in these dishes. Likewise each strain was planted in Petri dishes with the New York strain, and also all three strains were planted in the same dish. Dark lines developed at the juncture of the colonies in each case, but perithecia were produced only where the New York strain touched the other strains and in concentric circles about the center of inoculation in the colonies of this strain (pl. 1, B).

Numerous solid media and decoctions were used in the attempt to obtain sporulation in the Oregon strain, but none of these gave positive results. Hydrogen-ion concentration, temperature, light, moisture, and aeration also were varied, but with negative results. Possibly these cultures are sterile, but the writer is inclined to think that if the right conditions were found they would sporulate. Both the Oregon strain and the Arkansas strain were isolated from ascospores. This, together with the fact that they formed immature fruiting bodies at several times, would indicate that they would form mature perithecia under the proper conditions.

The New York strain fruited intermittently, but only after the writer had worked with it for several months. When perithecia formed they were abundant, but at times the fungus would go for several months without fruiting. Then suddenly many of the fresh transfers would form perithecia. The uncertainty of perithecial formation was still more evident when a large number of plates and tubes were inoculated at one time. Although the greatest care was exercised to obtain uniform conditions in any one series, there would invariably be a few cultures which would not develop perithecia. Evidently there is some important factor influencing sporulation that has not yet been determined.

It is reported by Kirby (8) that all of the New York strains of *Ophiobolus graminis* isolated exhibit the phenomenon of heterothallism. He states that in order to get sporulation it is necessary to grow plus and minus strains together. However, under the conditions of the present work the New York strain, which came originally from Kirby, was always homothallic. Single ascospores were frequently isolated, and these were germinated and grown separately. These single-spore cultures produced mature perithecia repeatedly in Petri-dish cultures without the influence of mycelium from any other strains.

Further, when portions of mycelium from a monosporous culture were placed on opposite sides of a Petri dish containing string-bean agar, a line of dark-colored mycelium was formed where the two mycelia came together, and perithecia were developed along this line (pl. 2, A). In this case, two colonies from the same ascospore acted in the same manner as mycelia from the so-called plus and minus strains reported by Kirby (8). This experiment indicates that two advancing masses of hyphae may change the nature of the substrate to the extent that sporulation may be aided, and in this case induced along the line of contact of the colonies. In an environment that is not favorable for sporulation, or when weak cultures of the fungus are used, two strains may induce sporulation where otherwise they would not fruit. It is evident from these results that nutrition or the products of metabolism may act as a stimulant for sporulation. Possibly this is the explanation of the results obtained by Kirby. It is the opinion of the writer that the lack of sporulation of the Oregon and Arkansas strains of the fungus in the present studies is probably caused by their extreme lack of vigor. Due to their long culture on artificial media, with mycelium used for transfers, their reproductive ability probably became so low that the various stimuli used could not restore it. It seems entirely possible that when other factors which influence sporulation are determined these cultures might be made to sporulate.

INFLUENCE OF TEMPERATURE ON GROWTH OF THE FUNGUS

The parasite was grown at different temperatures on 2 per cent potato-dextrose agar in Petri dishes, and the growth was determined by measuring the diameter of the colonies. The agar used had a hydrogen-ion concentration of 7 P_H , and in all cases the same amount of medium was used in equal-sized Petri dishes. Young, vigorous stock cultures growing in Petri dishes were employed for supplying the transfer material. In order to insure uniformity among all the transfers, blocks of agar 1 mm. square containing mycelium were cut at equal distances from the center of the stock colony and placed in the center of each Petri dish filled with fresh agar. The dishes

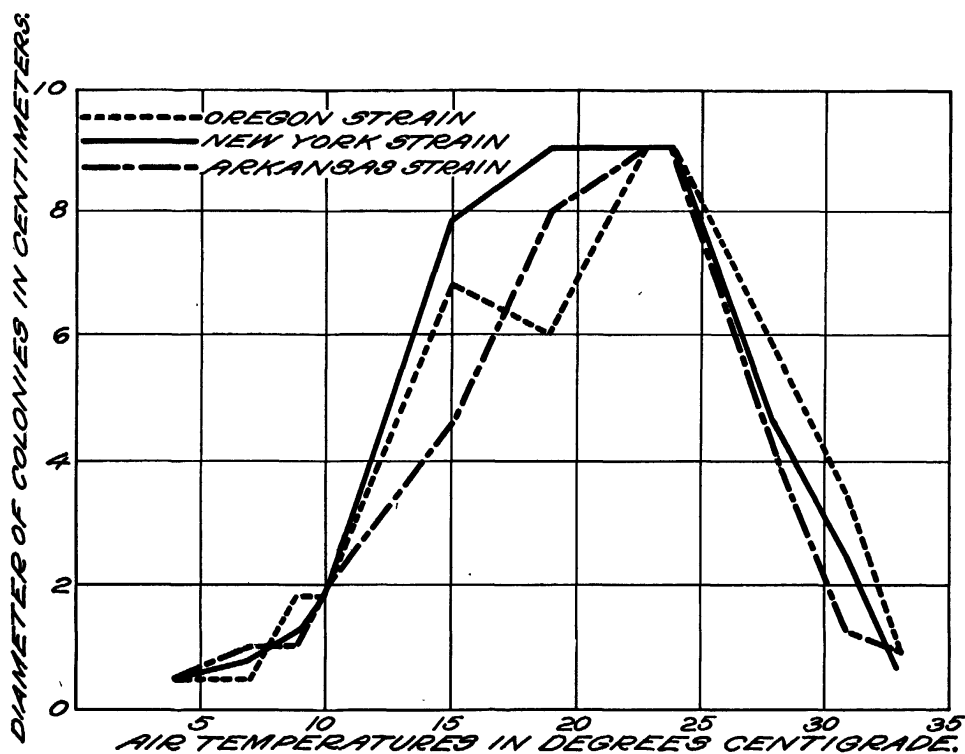


FIG. 3.—Influence of air temperature on the growth of mycelium of three strains of *Ophiobolus graminis*. Data from Table I

were then placed in the various temperature compartments of an Altmann incubator. The chambers of the incubator were kept as nearly as possible under the same conditions, except temperature. These chambers excluded all light when closed, hence the growth was in darkness. An exception to this was necessary in the case of the highest temperature in the first four experiments and in the case of the 28° C. temperature in the fifth experiment. These high temperatures were maintained in a separate incubator which contained a dim electric light. As this light did not strike the plates, directly, and as it was determined, as reported later in this paper, that light of that intensity had very little inhibitory influence on mycelial growth, it seems evident that this weak illumination did not impair the value of the temperature results obtained in this case.

Ten series with the Oregon strain, and four series each with the New York and Arkansas strains, were studied. In each experiment

there was a gradual increase in growth from the minimum temperature to the optimum, then a more rapid decrease as the maximum temperature was approached. A temperature of 36° C. was available in only the first four experiments, but growth of all cultures kept at this temperature in these experiments was entirely checked. The lowest temperature employed was 4° C., at which growth was exceedingly slight. Measurements of diameter of colonies of the different strains grown at the different temperatures are given in Table I and the averages are shown graphically in Figure 3.

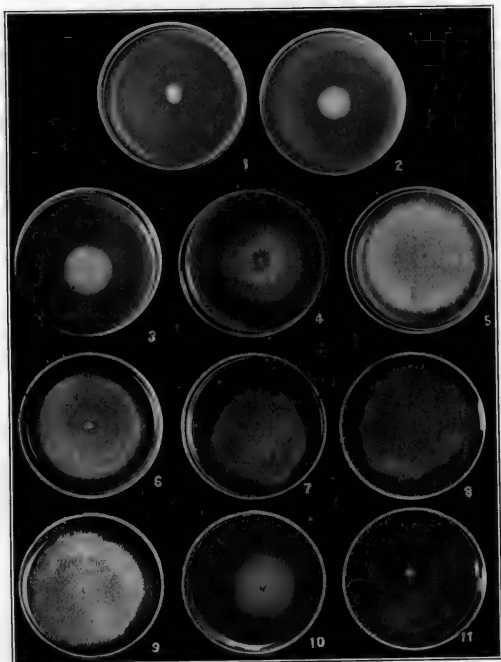
TABLE I.—Effects of air temperatures on the growth of the mycelium of three strains of *Ophiobolus graminis* on potato-dextrose agar, as shown by measurements of diameter of colonies

Strain of fungus	Diameter of colonies grown at the different temperatures										
	4° C.	7° C.	9° C.	10° C.	15° C.	19° C.	23° C.	24° C.	28° C.	31° C.	33° C.
Oregon -----	<i>Cm.</i> 0.5 .5 .5 -----	<i>Cm.</i> 0.5 .5 .5 -----	<i>Cm.</i> 2.0 2.0 1.5 -----	<i>Cm.</i> 2.0 2.0 1.5 -----	<i>Cm.</i> 6.5 7.0 7.0 6.5 -----	<i>Cm.</i> 6.0 6.0 6.0 6.0 -----	<i>Cm.</i> 9.0 9.0 9.0 9.0 -----	<i>Cm.</i> 9.0 9.0 9.0 9.0 -----	<i>Cm.</i> ----- ----- ----- 4.5 -----	<i>Cm.</i> 2.5 2.5 4.0 4.5 -----	<i>Cm.</i> 1.0 ----- ----- ----- -----
Average -----	.5	.5	1.8	1.8	6.8	6.0	9.0	9.0	-----	3.4	1.0
New York -----	.5 .5 .5 -----	1.0 1.0 .5 -----	1.5 1.5 1.0 -----	2.0 2.0 1.5 -----	8.0 8.0 8.0 7.0 -----	9.0 9.0 9.0 9.0 -----	9.0 9.0 9.0 9.0 -----	9.0 9.0 9.0 9.0 -----	6.5 4.0 3.5 -----	2.5 2.5 2.5 2.0 -----	.5 .5 1.0 -----
Average -----	.5	.8	1.3	1.8	7.8	9.0	9.0	9.0	4.7	2.4	.7
Arkansas -----	.5 .5 .5 -----	1.0 1.0 1.0 -----	1.0 1.0 1.0 -----	2.0 2.0 2.0 -----	5.0 5.0 4.0 4.0 -----	8.0 8.0 8.0 8.0 -----	9.0 9.0 9.0 9.0 -----	9.0 9.0 9.0 9.0 -----	4.0 4.5 ----- -----	2.0 2.0 .5 .5 -----	1.0 1.0 1.0 .5 -----
Average -----	.5	1.0	1.0	2.0	4.5	8.0	9.0	9.0	4.3	1.3	.9

A typical complete series of cultures is shown in Plate 3. It is evident that *Ophiobolus graminis* will grow at temperatures ranging from 4° to 33° C., with a maximum growth at 23° to 24°. This range of temperature for the growth of the fungus is almost as wide as the range for growth of the wheat plant, and even though wheat will grow at a slightly higher temperature, it can not be grown economically under such conditions. The optimum temperatures for the growth of both are about the same, as shown by McKinney and the writer (11) but it can not be assumed from this that the fungus is most destructive to wheat plants at those temperatures.

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON GROWTH OF THE FUNGUS

Growth for the three strains of the organism as related to hydrogen-ion concentration was studied in potato-dextrose agar, nutrient broth, and Czapek's full nutrient solution. In the solid medium, growth rates were determined by measuring the diameter of the colonies. In the liquid media, the dry-weight method was used. The hydrogen-ion concentrations of the media were determined by the Clark and Lubs (2) colorimetric method. The determinations were made at room temperature, following adjustment of the media to a desired reaction, and such values represent the initial hydrogen-



Petri-dish cultures of *Ophiobolus graminis* on 2 per cent potato-glucose agar, incubated at different temperatures as follows: 1, 4° C.; 2, 7°; 3, 9°; 4, 10°; 5, 15°; 6, 19°; 7, 23°; 8, 24°; 9, 28°; 10, 31°; 11, 33°



A.—Goldcoin wheat plants 30 days old, showing a severe case of take all. These plants were grown under greenhouse conditions in soil inoculated with a pure culture of the Oregon strain of *Ophiobolus graminis*

B.—Healthy plants of same age and same variety, grown in uninoculated soil

ion concentration. In the solid medium, only initial determinations were made, but in the liquid media both initial and final PH values were determined. As soon as possible after adjusting the media they were inoculated with a uniform quality and amount of inoculum, namely, young, vigorous mycelium on blocks of agar 1 mm. square.

Potato-dextrose agar with hydrogen-ion concentrations in rather regular steps from P_H 3.0 to P_H 10.0+ was used. The media were made as usual, except only one-half the required quantity of water was added at first. Equal quantities of the concentrated agar were placed in the flasks, each of which was plugged, properly labeled, and sterilized in the autoclave for 40 minutes at 8 pounds pressure. The quantities of acid or alkali necessary to bring the medium in

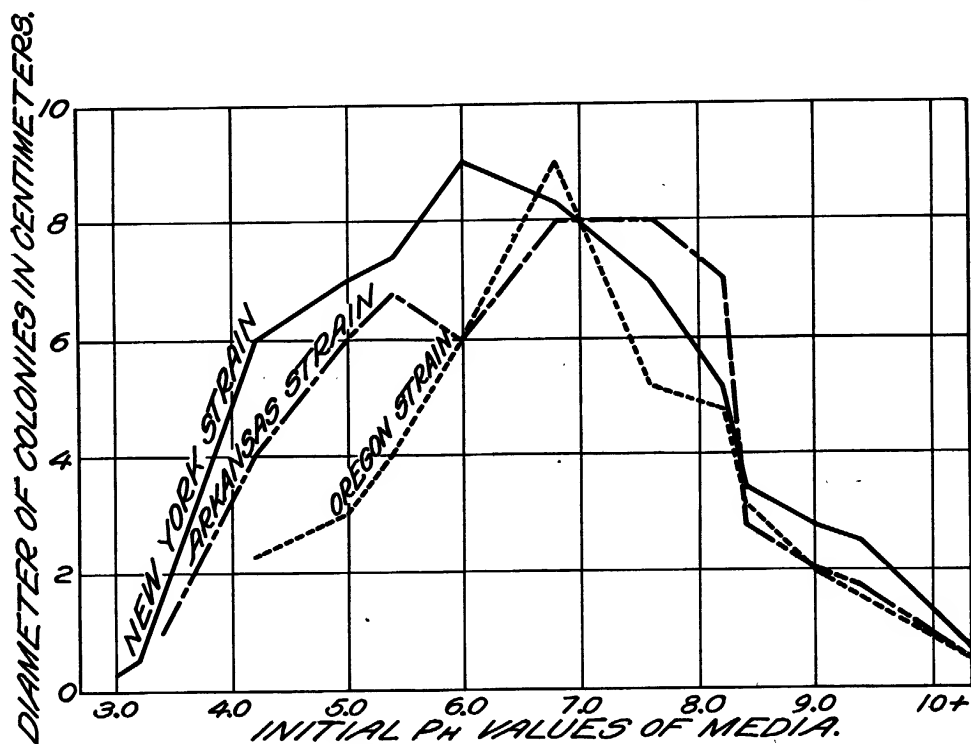


FIG. 4.—Influence of hydrogen-ion concentration of potato-dextrose agar on the growth of mycelium of *Ophiobolus graminis*. Data from Series 3, a-f, Table II

each flask to the desired reaction was calculated and the addition subsequently made. Calculated amounts of water were then added to the flasks to bring the medium in each to the proper dilution. This method allowed adjustment of media to various hydrogen-ion concentrations by the addition of either acid or alkali without materially affecting concentrations and ratios of the nutrients.

Equal quantities of the agar were then poured into Petri dishes, which were properly labelled. Sufficient agar remained in each of the stock flasks for hydrogen-ion determinations, which were made immediately at room temperature. Each agar-poured plate was inoculated as just described and then incubated under glass bell jars in diffused light at room temperature. At the end of seven days, at which time the largest colonies nearly covered the agar surfaces, the diameter of the colonies was measured. The resulting data are given in Table II, and the averages from Series 3, a-f, are shown graphically in Figure 4.

TABLE II.—Influence of hydrogen-ion concentration of potato-dextrose agar on the growth of the mycelium of three strains of *Ophiobolus graminis*, as shown by measurements of diameter of colonies

Initial PH value of agar	Diameter of colonies in centimeters											
	Oregon strain						New York strain				Arkansas strain	
	Series a	Series 1b	Series 1c	Series 2a	Series 2b	Series 2c	Series 3a	Series 3b	Series 3c	Series 3d	Series 3e	Series 3f
3.0							0.0	0.0	0.0	0.5	6.0	0.0
3.2							.0	.0	.5	.5	.0	.0
3.4							.0	.0	2.0	1.0	1.0	1.0
3.8	0.5	0.5	0.5									
4.0	1.5	1.5	1.0									
4.2	2.0	2.5	3.0				2.0	2.5	6.0	6.0	4.5	3.5
4.4	3.0	3.5	3.5									
4.6	5.0	6.0	4.5									
5.0				3.0	3.0	3.0						
5.2				5.5	6.5	6.5	3.0	3.0	7.0	7.0	.0	6.0
5.4	6.5		6.5									
5.6								4.0	8.0	7.0	7.0	7.0
5.8	7.0	7.0	7.0									
6.0	6.5		7.0	6.0	7.0	7.0						
6.2							6.0	6.0	9.0	9.0	6.0	6.0
6.4	6.5	6.0	6.0									
6.6				7.0		5.5						
6.8							9.0	9.0	8.0	8.5		8.0
7.2				3.0	3.5							
7.4	4.0	4.0	3.0									
7.6							4.5	6.0	6.0	8.0		8.0
7.8	3.5	2.5	2.0									
8.0	1.5	2.0	1.5		2.5	2.5						
8.2	4.5	4.5	6.0				4.5	5.0	5.5	5.0	7.0	7.0
8.4	4.0	4.0	4.5	3.0	3.0	3.0	3.5	3.0	4.0	3.0	2.5	3.0
8.8	7.5	7.0	7.0	3.0	2.5	2.0						
9.0	3.5	2.0	4.0				2.0	2.0	3.0	2.5		2.0
9.2				3.0								
9.4								1.5	3.0	2.0	1.5	2.0
9.6				3.0	1.0	1.0						
10+				1.5			.5	.5	.5	1.0	.5	.5

It will readily be seen from these results that the three strains of the fungus varied somewhat in the amount of growth at the various hydrogen-ion concentrations. With the Oregon strain, the highest acidity allowing growth was P_H 3.8 in potato-dextrose agar. Growth increased rather regularly with decrease in acidity until a maximum was obtained at from P_H 5.6 to P_H 6.8. Beyond this, as the acidity diminished, mycelial growth of this strain decreased until it was checked at P_H 10.0+. The Arkansas strain exhibited growth at a concentration of P_H 3.4 and reached its maximum growth at P_H 6.8 to P_H 7.6. The most alkaline reaction permitting growth of this strain was just above P_H 10.0. The New York strain grew at all concentrations from P_H 3.0 to P_H 10.0+, with maximum growth at P_H 6.0.

In addition to the experiments with potato-dextrose agar, two experiments were conducted with the Oregon strain of the fungus in nutrient broth and Czapek's full nutrient solution. The results are given in Table III and represented graphically in Figure 5. In both, growth occurred at all reactions employed. However, the nutrient broth proved to be a more favorable medium at all hydrogen-ion concentrations than did the Czapek's full nutrient solution. In fact, the latter medium was found to be decidedly unfavorable for making these studies. Growth was comparatively feeble in the most

extremely acid and alkaline cultures, thus indicating that the concentrations necessary for inhibition lay slightly beyond the hydrogen-ion concentrations used in the experiment, which were P_H 4.8 to P_H 10.0+ for nutrient-broth growth and P_H 4.6 to P_H 10.0+ for Czapek's solution. In nutrient broth growth was best in cultures with an initial hydrogen-ion concentration of P_H 6.6. In Czapek's solution the greatest growth also occurred near this point. However, there was no striking optimum in the case of this medium. Final hydrogen-ion determinations of the media showed that all of the cultures involving Czapek's full nutrient solution became more acid with the growth of the fungus. The acid and slightly alkaline cultures of the nutrient broth shifted their reactions to greater alkalinity, while the most alkaline cultures tended to become slightly less alkaline. Unfortunately, no final determinations were made on the checks, so that it is impossible to say whether or not the latter changes were due to the growth of the organism or merely to changes in the reactions on standing.

TABLE III.—Influence of hydrogen-ion concentration of nutrient broth and Czapek's solution on the growth of the mycelium of the Oregon strain of *Ophiobolus graminis*

Nutrient broth				Czapek's solution			
Initial P_H	Final P_H	Growth	Average growth	Initial P_H	Final P_H	Growth	Average growth
		Gm.	Gm.			Gm.	Gm.
4.8	5.4	0.033	-----	4.6	4.2	0.018	-----
4.8	6.4	.045	0.039	4.6	4.2	.020	0.019
5.0	8.4	.100	-----	6.0	5.4	.007	-----
5.0	8.4	.105	.102	6.0	5.4	.010	.0085
5.4	8.4	.090	-----	6.4	5.8	.025	-----
5.4	-----	-----	.090	6.4	5.8	.020	.0225
6.6	8.6	.120	-----	6.4	6.0	.030	-----
6.6	8.6	.120	.120	6.4	6.0	.035	.0325+
6.8	8.8	.095	-----	6.4	6.2	.025	-----
6.8	8.6	.105	.100+	6.4	6.2	.035	.030
8.0	8.8	.085	-----	7.8	7.6	.020	-----
8.0	8.8	.095	.090	7.8	7.8	.020	.020
10.0	8.8	.040	-----	9.4	8.0	.020	-----
10.0	8.8	.055	.0475	9.4	8.0	.025	.0225
10+	8.8	.050	-----	10+	6.4	.020	-----
10+	8.8	.070	.060	10+	6.0	.015	.0175
10++*	9.2	.025	-----	10++	8.2	.015	-----
10++	9.2	.035	.030	10++	8.2	.020	.0175

* In the alkaline range, more NaOH was added to each succeeding flask; therefore, as one reads these figures, each 10+ had more chemical added than the previous one.

Although there are certain variations in the results in Table III, a few general conclusions may be drawn. The ranges of hydrogen-ion concentration in which *Ophiobolus graminis* will grow favorably are about the same for potato-dextrose agar, nutrient broth, and Czapek's solution. Growth took place in all cases where cultures possessed an alkaline reaction of or greater than P_H 10.0+, but it was very slight. This value, then, could be stated as approximately the most alkaline reaction in which vegetative growth of *Ophiobolus graminis* occurred. On the other hand, the most acid reaction permitting growth varied from P_H 3.0 and P_H 3.8, depending on the strain of the fungus. In these experiments the optimum hydrogen-ion concentration for the different strains varied too much to draw definite conclusions, except that if an average were taken it would occur on the acid side of neutrality.

These results vary somewhat from those obtained by Kirby (7). He obtained maximum growth of the New York strain on potato agar at a concentration of about P_H 9.0, and growth was inhibited at about P_H 3.2. The latter figure agrees very closely with the writer's results. However, the hydrogen-ion concentration for optimum growth obtained by Kirby is considerably more alkaline than that obtained in the present studies. Using corn-meal agar as a substratum he found the maximum growth at about P_H 9.2. The writer's cultures were kept in the light, while those studied by Kirby were kept in the dark. This may be a factor of importance. However, the writer is convinced that the strains of the fungus which he has been studying will grow better, in the media employed, at less alkaline reactions than those reported by Kirby. In this connection it may be said that Kirby (7) obtained less disease in wheat



FIG. 5.—Influence of hydrogen-ion concentration of nutrient broth and Czapek's solution on the growth of the mycelium of *Ophiobolus graminis*. Data taken from Table III

plants when the soil in which they were growing was made acid by addition of certain fertilizers than when it was made alkaline, and likewise more disease was obtained in untreated soil than in limed soil. Kirby made no hydrogen-ion determinations on the soils treated in different ways; at least no data were published to this effect, so it is impossible to correlate the data for the growth of the organism in pure culture with those for the occurrence and intensity of the disease of plants growing in such soils. These were only preliminary studies, but it is evident that more work is necessary on this phase of the problem.

INFLUENCE OF LIGHT ON GROWTH AND SPORULATION OF THE FUNGUS

Cultures of *Ophiobolus graminis* in Petri dishes kept under glass bell jars in the diffuse light of the laboratory grew slightly less than cultures kept under darkened bell jars, as shown in Table IV. Al-

though the difference in the rate of growth of these two sets of cultures was only slight, it was generally constant. The variation in temperature between the two groups of cultures was never more than 1 degree, and as the studies on the influence of temperature on the rate of growth of the fungus show that differences of 1 degree or less make little difference in the rate of growth, it is concluded that the decrease in growth of the cultures placed in the light was due to the influence of light and not to that of temperature.

TABLE IV.—*Influence of light on the growth of mycelium of Ophiobolus graminis*
[Data based on 7-day-old cultures]

Strain of fungus	Agar medium used	In light or dark	Diameter of colonies in centimeters							
New York	Potato-dextrose	Light.....	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
	do.....	Dark.....	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
	String bean.....	Light.....	7.0	8.5	7.0	7.0	7.0	7.0	7.0	7.0
	do.....	Dark.....	7.0	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Oregon.....	do.....	Light.....	4.0	4.5	4.0	4.0	4.0	4.5	4.5	4.0
	do.....	Dark.....	4.0	5.0	4.0	4.0	4.5	4.5	5.0	5.0
	do.....	Light.....	2.5	2.5	3.5	3.0	2.5	2.5	3.0	3.0
	do.....	Dark.....	3.0	4.0	3.5	3.0	3.0	3.5	4.0	4.0

The influence of light on sporulation was very marked in contrast to its influence on the growth of mycelium. With string-bean agar as a substratum, no culture kept in absolute darkness formed even a single perithecium, while most of those kept in the light formed fruiting bodies abundantly, as shown in Table V. One hundred and ninety-six cultures were observed at three different times and in all cases only mycelium was produced in those incubated in the dark. However, a few of the cultures kept in the light did not sporulate, which indicates that some influencing factor favorable for sporulation remains yet to be determined. The writer does not infer that perithecia will form only in the presence of light, for they occasionally are found on the roots of plants near the crown where they are formed in darkness, but from the laboratory experiments cited it is apparent that light favors the development of perithecia on the culture media used and under the conditions described.

TABLE V.—*Influence of light on the development of perithecia of Ophiobolus graminis*
[Data based on 47-day-old cultures]

Experiment No.	In light or dark	Total number of cul- tures	Number of cul- tures produc- ing peri- thecia
1.....	{Light.....	20	14
	{Dark.....	20	0
2.....	{Light.....	60	46
	{Dark.....	40	0
3.....	{Light.....	36	30
	{Dark.....	20	0

PATHOGENICITY OF THE FUNGUS

All three strains of the fungus used in these studies were pathogenic on wheat plants. To prove this, disinfected Goldcoin wheat was sown in soil that had been disinfected by steam and subsequently

inoculated with the different strains of *Ophiobolus graminis*. The soil used was a fine silt loam, as previously described, and was placed in an autoclave for 2 hours under 15 pounds steam pressure. Equal amounts of this soil were placed in water-tight metal cans 6 inches in diameter and 9½ inches deep. The upper 3 inches of soil was then removed from each can, and kept separate. To each portion of this soil was added 100 gms. of inoculum, which consisted of the fungus growing on cooked oat and barley kernels, as described previously. Each portion of soil and inoculum was then mixed together very thoroughly and placed in the proper can. The control cans were similarly filled with their portions of soil and 100 gms. of the uninoculated oats and barley medium. The wheat seed was sown 1½ inches deep in this uniformly inoculated layer. After sowing, all the soil cans were immediately placed in constant-temperature water baths in a greenhouse. Samples of the seed used in the experiment were disinfected with 1 to 1,000 mercuric chloride, washed in sterile water, and then placed on agar in Petri dishes. In all cases the kernels were found to be free from seed-borne fungi.

The plants growing in the inoculated soil soon became diseased, and later many of them died. In all cases the check plants remained healthy and the roots were white and disease-free, as indicated in Table VI and Plate 4. Microscopic examination showed the presence of the fungus in the tissues of the infected plants, and a pure culture of the organism was isolated from the attacked seedlings in the early stages of the disease. Dark-brown lesions developed on the roots and culms of all of the infected plants, and many of the leaves turned yellow and died. Several experiments similar to the above were performed later in the course of other studies, and similar results were obtained. All three strains of the fungus were found to be readily pathogenic, but the New York strain was more virulent and killed a greater percentage of plants than either of the other strains. This was possibly due to the fact that the New York strain fruited rather freely, making it possible to use ascospore cultures for transfers.

TABLE VI.—Pathogenicity of *Ophiobolus graminis* on Goldcoin wheat grown in steamed soil, inoculated with pure cultures of the parasite

[The controls were uninoculated]

Strain of fungus	Number of plants	Condition of plants *
New York.....	150	All plants diseased.
Control.....	50	No disease.
Oregon.....	150	All plants diseased.
Control.....	50	No disease.
Arkansas.....	150	All plants diseased.
Control.....	50	No disease.

* The disease was most severe in the pots inoculated with the New York strain. Plants grown in the pots inoculated with the Oregon strain showed a less severe attack, but they were more severely diseased than the plants grown in the pots inoculated with the Arkansas strain of the parasite.

It was also proved, under aseptic conditions, that *Ophiobolus graminis* caused the take-all disease of wheat. Wheat plants were grown on Crone's agar in glass culture tubes 2½ inches in diameter

and 20 inches long and inoculated with the fungus. Prior to planting and inoculating, the tubes containing the medium were plugged with cotton and sterilized at 15 pounds pressure for 40 minutes. After the agar solidified, disinfected Goldcoin wheat seeds which had been previously germinated for 5 days on potato agar in Petri dishes were planted in the large tubes. The previous germination was carried out to insure seedlings that were free from fungi and bacteria. The seed was handled under aseptic conditions throughout the whole process previous to inoculation. Two days after planting the seed, a block of agar containing mycelium of a single strain of *O. graminis* was inserted in each tube, except the control tubes, which were handled similarly but not inoculated. All of the tubes were placed in the laboratory in such a position that they received equal illumination, and the plants were carefully watched for any indication of disease.

The fungus soon spread over the surface of the agar in the inoculated tubes, and in 4 days symptoms of the disease on the wheat seedlings began to appear. The characteristic dark lesions associated with take-all first started at or near the base of the roots. This discoloration then spread both up on the culm and farther down on the roots, and the leaves soon turned yellow and died. By the time the plants were 28 days old, all of the inoculated plants had died, while the uninoculated plants were still healthy and thriving. These latter had grown to the top of the tubes and were badly crumpled, due to their upward elongation being stopped by the plugs in the mouths of the tubes. A week later part of them had died, apparently due to lack of space and the drying out of the agar, but no disease lesions were visible. The results are given in Table VII.

TABLE VII.—*Pathogenicity of Ophiobolus graminis* on Goldcoin wheat grown under aseptic conditions on agar contained in culture tubes

[Inoculations were made with pure cultures of the parasite. The controls were uninoculated]

Strain of fungus	Number of tubes	Number of plants in each tube	Total number of plants dead in all the tubes	
			After 21 days	After 28 days
New York.....	4	3	12	12
Control.....	2	3	0	0
Oregon.....	4	3	0	12
Control.....	2	3	0	0
Arkansas.....	4	3	0	12
Control.....	2	3	0	0

Pure cultures of *Ophiobolus graminis* were obtained by putting bits of the culms of the infected plants grown in the culture tubes on fresh agar in Petri dishes. Plants from uncontaminated culture tubes invariably gave a pure culture of the parasite.

Delacroix (3) inoculated soil with a water suspension of ascospores of *Ophiobolus graminis* obtained from perithecia which had developed on diseased plants in the field. Wheat plants grown in this inoculated soil developed a rather high percentage of infection and

showed marked injury. Plants grown in the same manner and under the same conditions in uninoculated soil remained healthy.

Waters (15) found that pure cultures of this organism were pathogenic on wheat plants growing in tubes of sterilized soil to which a pure culture of *Ophiobolus graminis* had been added. Kirby (7) also found that wheat plants developed the characteristic symptoms of take-all when grown in soil inoculated with pure cultures of this fungus.

OVERWINTERING OF THE PARASITE

These studies were carried out with three kinds of material: (1) agar cultures of the parasite in ordinary test tubes; (2) artificially inoculated soil; and (3) diseased plant material. All of the experiments were conducted during the winter of 1922-23, which was unusually severe at Madison, Wis.

PURE-CULTURE STUDIES

All three strains of the parasite, as used in previous experiments, were cultured on potato-dextrose agar in test tubes. After a good growth of mycelium was obtained the cultures were placed outdoors in an exposed position throughout the winter. In the spring it was found that several tubes had burst, due to the freezing of the agar. Transfers of the mycelium from all of the overwintered tubes showed that all of the cultures were alive. In all cases vigorous cultures of the three strains were obtained.

STUDIES WITH ARTIFICIALLY INOCULATED SOIL

An experiment was conducted with three lots of soil contained in 6-inch metal cans. This soil was inoculated on October 18, 1922, with the mycelium of the New York, Arkansas, and Oregon strains of the parasite. Goldcoin wheat seed was planted at the time of inoculation. The plants showed signs of disease on November 22, 1922, and at this time they were removed from the soil as completely as possible, including the roots, and the soil was left out of doors throughout the winter. All of the plants removed from the soil were examined carefully for perithecia, but none were found. On March 26, 1923, Goldcoin wheat seed was again planted in all the cans. Early in May take-all developed in all of the 21 plants growing in the soil which had been inoculated with the Oregon strain of the parasite. The disease did not develop in plants growing in the soil inoculated with the New York or Arkansas strains and the disease was mild in the plants growing in the soil inoculated with the Oregon strain.

A second experiment was conducted in exactly the same manner and at the same time as the one just described, except that no seed was planted in the soil in the fall after inoculation. In the spring Goldcoin wheat seed was sown, and a severe case of take-all developed in all of the 21 plants growing in the soil which had been inoculated with the Arkansas strain of the parasite. No disease occurred in soil inoculated with the Oregon or New York strains of the fungus, and more occurred in the uninoculated controls in either experiment. It is not known just why certain strains of the fungus were killed during the winter. Possibly unevenness of snow cover may have been the cause.

A third experiment was carried out on a small plot of sterilized soil. This was divided into three parts, and each part was inoculated in the fall of 1922 with one of the strains of the parasite. Goldcoin wheat seed was sown in this soil in the fall and about 400 plants developed to the seedling stage, when all but 57 became diseased and died in the fall. Fifteen plants of those living were in the soil inoculated with the New York strain of the fungus, 28 in that inoculated with the Oregon strain, and 14 in that inoculated with the Arkansas strain. Plants growing in uninoculated control soil grew thriftily until cold weather came on. In the spring only 16 plants remained alive in the plots which had been inoculated, and these plants showed no signs of disease. All of the plants in the uninoculated control plot were winterkilled. Owing to the unoccupied space in the inoculated and uninoculated soils in the spring, it was decided to plant Marquis seed in both plots to determine whether the fungus was still living in the soil and pathogenic. This was done on April 27, and the 16 Goldcoin plants were allowed to remain in the inoculated plot. By May 17 all of the Marquis plants growing in the last-mentioned plot commenced to turn yellow and soon developed a severe case of typical take-all seedling blight. About 95 per cent of these plants died in a short time, while the plants growing in the uninoculated control soil developed normally.

The 16 overwintered Goldcoin wheat plants growing in the infested soil showed no signs of disease until the last of May, when they turned yellow and died. It is believed that the winterkilling of the Goldcoin control plants and the escape of the plants growing in the infested soil is explained on the basis of irregularities in snow drifting during the winter.

STUDIES WITH INFECTED PLANT TISSUE

Plant material obtained from the experiments dealing with host susceptibility to the New York strain of the parasite were employed in this study on overwintering. In one case five No. 00 ash pails (5 gallons capacity) containing infected stubble in infested soil were left outdoors over winter. This stubble was rooted in the soil, and the soil was not disturbed, except when stubble was removed for purposes of making examinations for perithecia. In another case the infected stubble was removed from the soil and placed in open earthen flowerpots. In both cases the material without protection was exposed to the winter weather.

Examinations were made on October 17, 1922, and perithecia were forming on the culms just above the crown in both lots of stubble. By January 3, 1923, asci had developed and many contained immature spores. Numerous paraphyses were also present. By April 1 the ascospores appeared mature, and those from one plant were tested for germination. It was found that 4.5 per cent of the ascospores germinated when placed in distilled water on a slide under a cover glass. The same plant with the remaining fruiting bodies was then placed outside in its previous location, and on May 1 this plant was brought into the laboratory with several others, and ascospores from all of them were tested for their viability as before. Spores from the plant previously tested still showed about the same percentage of germination, while spores from other plants gave practically 100 per cent germination. Some of these spores were obtained from fruiting

bodies which were produced 2 to 3 cm. above the soil line on plants still standing as in the soil, while others were from plants frozen in the bottom of the flowerpots where they had been placed.

From the foregoing evidence it is clear that *Ophiobolus graminis* will survive the winter in the mycelial and ascospore stages. Although there were cases when the fungus was killed during the winter, it is evident that, under certain conditions at least, the fungus can live through rather severe winter conditions.

AGE OF WHEAT PLANTS IN RELATION TO SUSCEPTIBILITY

In order to obtain data on the relation of age of wheat plants to susceptibility to *Ophiobolus graminis*, it was necessary to sow all of the seed used in any one experiment at one time and to apply the parasite at intervals throughout the development of the plants, or to plant the seed at intervals and inoculate the plants at one time. Both methods were used with satisfactory results. In this study four experiments (numbered I to IV) were conducted.

Experiment I was conducted out of doors in soil contained in No. 00 ash pails of 5 gallons capacity. Experiments II, III, and IV were performed in the greenhouse in Wisconsin soil-temperature-control tanks, and the temperature of the soil was held as near 18° C. as possible throughout the growth of the plants. In all of the experiments the water content of the soil was 55 per cent of the total water-holding capacity, and this percentage was maintained as constant as possible throughout the experiments by frequent weighings. An excess amount of seed was always planted in the containers, and after the plants had reached about the third-leaf stage they were thinned to a uniform number. The inoculum used in these experiments consisted of the mycelium of the New York strain of the parasite growing on the barley and oat-kernel medium previously described.

In the series sown at one time the first inoculation was made when the seed was planted. In the series sown at intervals the whole series was inoculated at the time the last planting was made. Growing plants were inoculated by carefully removing the upper 2 inches of soil from the pots, great care being taken not to cause undue injury to the roots. The mycelium of the parasite growing on the barley and oat-kernel medium was then added to the soil, the whole mass was well mixed and returned to the pots and carefully worked around the roots. Great care was taken to use cultures showing the same amount of growth and to add the same amount of medium to all the pots inoculated. In the control pots, in each case, an equivalent amount of sterile barley and oat medium was added to the soil in the same manner.

In Experiments I and II, California Club wheat was used, and the seed was sown at one time. The inoculations were made at intervals of 2 weeks after sowing, the first inoculation being made in one set at the time of sowing. As shown in Table VIII, all of the inoculated plants in these two experiments developed take-all and all eventually succumbed. All of the plants grown in the uninoculated soil remained healthy and matured normally. The amount of infection produced in one of these experiments is shown in Plate 5. The plants from seed which was inoculated at the time of planting began to turn yellow soon after emergence, and most of them died while in the seedling stage.

TABLE VIII.—*Period of susceptibility of California Club wheat plants to the attack by Ophiobolus graminis in Experiments I and II*

[The seed was sown at one time and the inoculum was applied at intervals during the growth of the plants. Controls were uninoculated]

Number of days after planting before inoculum or media was added	Treatment of plants	Number of diseased plants					
		Experiment I (out of doors)			Experiment II (in greenhouse)		
		Number of plants	Number of plants badly diseased	Number of plants slightly diseased	Number of plants	Number of plants badly diseased	Number of plants slightly diseased
Same day.....	Inoculated..	20	20	0	7	7	0
Do.....	Control.....	10	0	0	7	0	0
14.....	Inoculated..	20	0	20	7	0	7
14.....	Control.....	10	0	0	7	0	0
28.....	Inoculated..	20	0	20	7	0	0
28.....	Control.....	10	0	0	7	0	7
42.....	Inoculated..	20	0	20	(a)	0	0
42.....	Control.....	10	0	0		0	0
56.....	Inoculated..	20	0	20		0	7
56.....	Control.....	10	0	0	7	0	0
70.....	Inoculated..	20	0	20			
70.....	Control.....	10	0	0			

• Plants destroyed by rats.

• Plants were in bloom.

In Experiments III and IV, Goldcoin seed was sown at intervals of two weeks. The inoculations were made at the time of the last planting. As in Experiments I and II, all the inoculated plants became diseased, while the control plants remained healthy, as indicated in Table IX. The plants from seed sown at the time of inoculation developed the disease first and showed the most severe injury. Many of them died in the seedling stage, and none of them matured seed. Likewise the plants that were 2 weeks old when inoculated were attacked in a similar manner, but to a less degree. However, the plants that were older when inoculated did not show the effects of the disease until later.

TABLE IX.—*Period of susceptibility of Goldcoin wheat to attack by Ophiobolus graminis in Experiments III and IV*

[Plants grown under greenhouse conditions, in soil held near 18° C. The seed was sown at intervals and the inoculations were made at the time of the last planting. Controls were not inoculated]

Age of plants when inoculum or media was added (days)	Treatment of plants	Number of diseased plants					
		Experiment III			Experiment IV		
		Plants grown from June 27 to Sept. 12			Plants grown from Sept. 7 to Dec. 6		
		Number of plants	Number of plants badly diseased	Number of plants slightly diseased	Number of plants	Number of plants badly diseased	Number of plants slightly diseased
Just planted.....	Inoculated..	30	0	0	30	30	0
Do.....	Control.....	10	0	0	10	0	0
14.....	Inoculated..	30	30	30	30	15	15
14.....	Control.....	10	0	0	10	0	0
28.....	Inoculated..	30	30	30	30	0	30
28.....	Control.....	10	0	0	10	0	0
42.....	Inoculated..				30	0	30
42.....	Control.....				10	0	0

CEREAL INVESTIGATIONS.

That all of the inoculated plants of the four experiments became diseased, regardless of their age at the time of inoculation, is very significant. In two of the experiments part of the plants were in the flowering stage at the time of their inoculation, but these plants showed distinct lesions produced by the fungus, and by October *Ophiobolus perithecia* had formed on them. The same was true with the plants inoculated earlier, except that these were attacked more severely.

From these results it is evident that the varieties of wheat studied are susceptible to attack by the fungus during their entire growing period. The disease is most severe during the seedling stage of the host, and only a small portion of the plants attacked at this stage ever recovered. Whether, under field conditions, they would recover sufficiently to produce a crop is not known, but reports from field observations make this seem improbable (13, 9). Plants infected during the late stages of development, or plants recovering from earlier infections, produce symptoms known as "white heads." (1)

Although the inoculation method used in these experiments caused considerable root mutilation, it is believed that this does not seriously affect the results obtained. This is supported by the fact that the most severe cases of disease always occurred in the plants grown in the soil which was inoculated at the time of sowing the seed. Furthermore, results discussed later show that the fungus hyphae readily penetrate the unbroken epidermis of the roots.

PARTS OF WHEAT PLANT SUSCEPTIBLE

Preliminary studies to determine what parts of the wheat plant are susceptible to attacks of *Ophiobolus graminis* were carried on in large glass tubes containing cultures of the fungus. When the surface of the medium was thoroughly covered with mycelium, a 4-inch layer of cooled Crone's plant agar was poured over the colony. Wheat seedlings, previously germinated under sterile conditions, were then placed in the tubes, and the cultures were placed in a light place in the laboratory. Plants for experimental controls were grown in the same manner, except that no fungus was in the tubes.

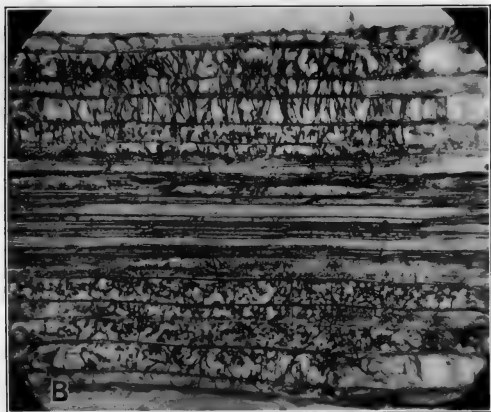
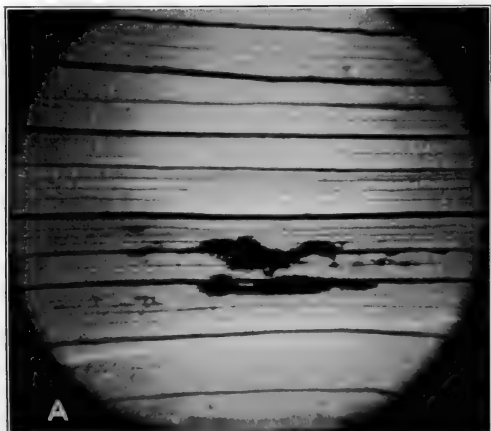
The roots of the seedlings were the first parts to be attacked. In from two to three days after planting, dark lesions began to show on the roots, and soon the entire roots were blackened. The culms did not show any signs of attack until four to five days after the roots had shown symptoms of invasion. The control plants remained entirely healthy.

In studying the histology of diseased tissues, as reported later, the fungus was found to enter the roots at any point when the plants were grown in the soil. Mycelial threads could be seen entering through the epidermis at various points along young roots, except near the distal ends where they were elongating rapidly. Single lesions also were noted on roots that had healthy tissues for several centimeters above and below the diseased portion. These observations, together with the fact that the roots could be infected while the rest of the plant was free from the fungus, definitely prove that the roots are susceptible to attack by *Ophiobolus graminis*.



Healthy (A) and diseased (B, C, D, E) California Club wheat plants, 89 days old, grown in the greenhouse. The soil in which the diseased plants were grown was sterilized and then inoculated with mycelium of the New York strain of *Ophiobolus graminis*

- A.—Healthy plants grown in sterilized uninoculated soil
- B.—Diseased plants grown in sterilized soil inoculated at the time of sowing
- C.—Same as B, except not inoculated until 14 days after sowing
- D.—Same as B, except inoculated 28 days after sowing
- E.—Same as B, except inoculated 56 days after sowing



A.—Photomicrograph of an isolated lesion on the sheath of a Goldcoin wheat plant artificially inoculated and grown in the greenhouse. $\times 16$
 B.—Photomicrograph of a longitudinal section of a wheat root, artificially inoculated and grown in the greenhouse, showing the cells of the cortex filled with the mycelium of *Ophiobolus graminis*. $\times 160$

An experiment similar to the one just described was conducted on the culms. For this purpose the plants were grown in tubes containing Crone's agar. When the plants were about 4 inches tall, cooled potato-dextrose agar was poured into the tubes to a depth of one-half inch. *Ophiobolus* was placed in some of these tubes, and others were left uninoculated for controls. The culms began to turn dark at the surface of the agar soon after the mycelium had spread over the medium and had come in contact with the culm tissues. The darkening progressed up and down the culm, and as the agar dried away from the surface of the walls of the tubes, the fungus grew down through this opening and attacked the roots. The fungus spread through the agar to a very slight extent. As in the case of the roots, it was found that the fungus penetrates the leaf sheaths and culms at any point below the surface of the soil. Often isolated lesions were noted on the sheaths, thus indicating that the fungus had entered at that point (pl. 6, A).

The green leaves of 10 plants in the early heading stage growing in the field also were tested for susceptibility to infection. Rubber rings, with a diameter a little less than the width of the leaves, were placed on the surface. A water suspension of ascospores was placed in these and a cover glass placed over each ring. The rings were held in place on the leaves with fine spring clamps. No infection took place by this method of inoculation. A few of the spores sent out short germ tubes, but they never entered the leaf. This method seemed unsatisfactory and it was discontinued because the water evaporated too rapidly and left the germinating spores dry.

Numerous wheat heads grown in the field were dipped in an ascospore suspension and then covered with glassine bags. The bags were tied closely about the culm to prevent evaporation, and were allowed to remain in place for four days, after which they were removed. No sign of disease developed, and the heads matured good seed. Ten plants growing in pails in the greenhouse were thoroughly covered with a spray of ascospores and placed in a moist chamber. Part of the plants used were young, while others had headed out. The air of the chamber was kept saturated with moisture, and the temperature was held as near 20° C. as possible. The light conditions in the chamber were poor. After four days the plants were taken out of the chamber and placed in the greenhouse. No visible signs of disease appeared on the plants. Microscopical examinations showed that a number of the spores had germinated, but in no case had they penetrated the wheat plants. The plants were examined the day after they were removed from the moist chamber and at short intervals for several weeks, but no sign of disease appeared. Finally, bits of mycelium were placed on leaves and heads of 10 more plants. A needle was run through the masses of mycelium and into the tissues of the plant. The plants were placed in a moist chamber for four days under the same conditions as before. They were allowed to stand one day after being removed before being examined for infection. By that time the mycelium had dried up and none could be found in the tissues, except a few pieces pushed in with the needle. Later no mycelium could be found, and disease symptoms never developed.

From the results obtained in these experiments, indications are that the leaves and heads are not susceptible to attack by *Ophiobolus*

graminis. The writer feels fairly confident that the leaves and heads of wheat plants growing in the open field are seldom if ever attacked. The dying of the upper parts of the plant evidently is due either to the inability of the roots and culms to allow sufficient food materials and water to reach these parts or to the development of toxic substances in the regions of infection. The first view is the one which has been generally held for this type of phenomenon, but data are now available which indicate that toxic substances are given off by some fungi, and these toxins are capable of causing plants to wilt (4). Possibly both of these factors operate, but the former would appear to be the chief factor in this case, due to the great reduction of the root area and the partial clogging of the vessels.

HISTOLOGY OF DISEASED WHEAT PLANTS

Suitable material for the histological studies was obtained by killing and fixing diseased and healthy plants each day as the disease progressed. Virgin soil was thoroughly sterilized by steam pressure, then *Ophiobolus* mycelium, previously grown on cooked cereals, was thoroughly mixed with the soil. This was placed in small cans, and disinfected Goldcoin wheat seed was planted at a depth of 1 ½ inches. An equal number of uninoculated cans for growing control plants were also prepared in the same manner, except that no fungus was added to the soil. The cans were placed in constant-temperature tanks and the temperature of the soil was maintained at 12° C. The plants from an inoculated and an uninoculated can were carefully washed with water and the plants killed, embedded in paraffin, and later sectioned according to the usual technique. Various killing and fixing agents, as Flemming's medium, Gilson's, Carnoy's, and chrom-acetic solutions, were used. The material killed and fixed in Gilson's fluid gave the best results for general histology, but such material was very poor for showing the detailed structure of the host cells. Two series of such plants grown at separate times were prepared in the above manner.

While pursuing these studies the macroscopic symptoms also were carefully noted. The first primary root broke through the coleorhiza two days after planting and three days later this root appeared slightly dark and watersoaked near its base. The next day this water-soaked area had turned a dark brown to black, and on the eighth or ninth day after planting the primary roots had become so badly decayed that they easily broke off. In the meantime, as the other roots developed they were also attacked, and lesions developed which were similar to those occurring on the first primary root. Often a root showed several distinct and separate lesions, indicating that the fungus had penetrated at various points. The coleoptile emerged from the soil on the eleventh day and an examination of the lower part of this, next to the old seed, revealed a slight discoloration. The following day this portion of the seedling was dark brown, and the lesion was extending farther up.

On the fourteenth day after planting the seedlings began to show symptoms just above the ground line. Brown streaks were visible on the coleoptile near the soil, but they did not extend up the sheath. The infected plants were decidedly smaller than the control plants.

and at the second-leaf stage (15 days old) the first leaf began to turn yellow. Some plants showed this chlorotic condition before the first leaf appeared, but these were only occasional cases. The bright-green color of the leaf faded out, leaving a yellow color in its place. At times this area turned bronze instead of yellow. The discoloration usually started first at the tip of the leaf and extended down the blade, but it often started back of the tip or on one edge. Finally, the whole leaf yellowed and died and turned a light-brown color. In a very short time the second leaf also became discolored similarly to the first one and died. Some of the infected plants recovered and lived indefinitely. Usually the primary root system on these plants was gone and part of the permanent roots was badly decayed, but new ones were formed to replace them. In case the plant had sufficient vitality to form new roots as fast as the fungus killed the old ones it lived, but if its vitality was low and new roots were not formed it soon died. In this event the roots became entirely decayed and the culm turned a dull-black color for about a centimeter above the soil line.

The embedded material for the histological studies was sectioned 5 to 10 microns in thickness and stained in saffranin and light green. These stains proved satisfactory and enabled the writer to follow the fungus fairly readily. Often the mycelium grew along the surface of the tissues and formed a mat of hyphae before penetrating the cells. The mycelium penetrated the wall at any point. Occasionally there was a swelling of the hyphae on each side of the wall, with constrictions of the segments in the wall.

The fungus was found to enter the plant at any point below the surface of the soil. The coleorhiza was the first tissue found invaded, and the parasite often spread from this point into the developing root. However, the primary infection of the seedling took place chiefly by the fungus growing between the coleorhiza and the root, with subsequent penetration. As the root elongated, it became invaded at points below the primary infection, and in case the basal parts escaped infection, as they often did, the root was usually attacked at other points. After the fungus had entered the root it spread in all directions. However, usually the fungus did not spread lengthwise as much as it did toward the center of the root, as shown in Plate 6, B, because the root was being penetrated at various points along its entire length. On reaching the endodermis of the young root, the fungus stopped its inward growth and did not penetrate this until most of the cells of the cortex had been killed. Even after penetrating the central cylinder the fungus appeared to remain, for the most part, in the parenchymatous cells, and was found inside the bundles of only the old and very badly diseased plants.

The fungus invaded the leaf sheaths and culms in the same manner as the roots, penetrating at all points below the surface of the soil. The fungus was found in all the tissues of this region except the primordia. Frequently the coarse type of mycelium was found in the vessels of the lower portion of plants inoculated in large test tubes.

Badly infected plants are usually black at the base. This blackening may occur under the outer leaf sheath or it may show through

this sheath. Usually the blackening is due to a staining of the tissue by the invading fungus, or it may be due to the crusts of the mycelium.

Examinations show that the cytoplasm and nucleus begin to disintegrate as the fungus enters a cell. In fact, the nucleus was often seen breaking up before the fungus actually entered the cell, and by the time the hyphae were well distributed through the cells no sign of the nucleus could be seen. The cell wall was also affected by the presence of the fungus, but the exact nature of the change was not determined. On staining the tissues with saffranin and light green, the cellulose cell walls took up the light-green stain until the fungus entered the cell, or the adjoining cell, then the walls took up and retained the saffranin. It appears from this that enzymes or toxic substances penetrate the host cells in advance of the fungus. The fact that the cell contents of the host showed disintegration before any hyphae entered the cell also substantiates this view.

SUMMARY

The name *Ophiobolus graminis* Sacc. is used in this paper to designate the fungus causing the take-all disease of wheat.

Perithecia of the New York strain of this parasite were formed in pure cultures on artificial media and on wheat plants growing in soil that had been inoculated with the fungus. Ascospores and paraphyses were formed in abundance.

When conditions were favorable the ascospores germinated rapidly, sending out from 1 to 3 germ tubes which branched and rebranched, forming a network of hyphae. Numerous cultures of the New York strain, made from single ascospores, produced perithecia and mature ascospores, thus showing that this strain of the fungus is homothallic. The Oregon and Arkansas strains studied were never induced to sporulate.

Although the three strains of the parasite reacted similarly to temperature, they showed some variation in this regard. In all cases growth took place between 4° and 33° C., but it was very slight at the extremes. The optimum temperature for the growth of the New York strain was found to range between 19° and 24°, whereas the optimum for the Oregon and Arkansas strains was between 23° and 24°.

Light checked mycelial growth slightly and greatly stimulated sporulation in the New York strain of the parasite.

The strains of the parasite used in these studies grew within the P_H range of 3.0 to 10+. The optimum hydrogen-ion concentration for the New York strain was found to be P_H 6.0 and that of the Oregon strain was P_H 6.8. The optimum for the Arkansas strain was less limited, in that it extended between P_H 6.8 and 7.6.

The ascospores and probably the mycelium of the parasite were found to overwinter in plant refuse in the soil. Mycelium overwintered readily in agar cultures in test tubes.

The varieties of wheat used in these investigations (Goldcoin, California Club, and Marquis) were all found to be susceptible to infection by *Ophiobolus graminis* during the entire growing period of the plants. All of the studies made thus far show, however, that plants are most susceptible in the seedling stage.

Infection was never obtained in any part of the plants much above the soil line.

Histological studies were made from plants exhibiting various stages of infection and these showed that the parasite entered the unbroken epidermis of the underground portions of the leaf sheaths, culms, and roots. The parasite first destroys the cortex of the roots and later enters the central cylinder. It also destroys the leaf-sheath and culm tissues and later enters the vessels, but it does not appear to make much progress after it enters the vessels.

These studies indicate that the bleaching of the aboveground portions of plants is not due to a systemic infection of the whole plant, but to the cutting off of the food materials and water supply and possibly to the toxic substances.

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INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON INFECTION OF YOUNG WHEAT PLANTS BY *OPHIOBOLUS GRAMINIS*¹

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INTRODUCTION

There are many statements in the literature regarding the influence of weather and soil drainage on the occurrence of the take-all and foot-rot diseases of wheat. In most cases, however, these reports have been based on rather casual observations which, in many instances, have been made on the foot rots in general rather than on take-all, the specific foot rot caused by *Ophiobolus graminis*. Doubtless the lack of agreement among certain workers as to the influence of temperature and moisture on the take-all disease is due largely to the fact that they have been dealing not with one disease, but with several which are very similar in appearance but caused by different parasites.

Gaillot (6)³ states that in France wheat foot rot is favored by mild, moist weather, whereas Reuther (13) states that in Germany the foot disease was favored in 1913 by vernal frosts. In neither of these reports is it clear which foot rot is referred to, but it appears that these workers have in mind the foot rots as a whole. According to Lindau (10, p. 256) wet soil favors infection by *Ophiobolus graminis*, and directly in line with this Dombrovski (5) states that the proper drainage of the soil reduces losses due to this parasite.

McAlpine (11) states that opinions are most conflicting in connection with the occurrence of take-all in Australia. He states that some observers claim it to be more prevalent during wet seasons, while others say that dry seasons favor the disease. His own observations indicate that the disease occurs under all climatic conditions. Robinson (14), also working in Australia, states that a dry summer followed by a wet winter affords the most favorable conditions for the development of the disease. Sutton (16), working in western Australia, and Waters (17), working in New Zealand, claim that wet soil conditions favor take-all, whereas Hori (7), working in Japan, thinks the disease is less prevalent on poorly drained soils. In this connection, however, it should be pointed out that Hori's observations included a large number of cases of take-all on barley. It is entirely possible that the disease on this host may not react the same as on the wheat plant when placed under similar soil conditions.

In this country take-all has been observed for so short a time that little definite information is at hand concerning its development in the field under different temperature and moisture conditions. Kirby (9), working in New York, states that his observations indicate little or no difference in the amount of infection occurring on high

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² These investigations were carried on in cooperation with the Wisconsin Agricultural Experiment Station.

³ Reference is made by number (italics) to "Literature cited," p. 840.

and on low ground. He states, however, that in a few fields the infection appeared to be very much heavier on the lower and wetter land. Rosen and Elliott (15), working in Arkansas, also state that the disease seems to be favored by wet soil.

One of the writers (McKinney) has observed take-all under many different field conditions, and while the disease has appeared to be more severe in low undrained portions of some fields, this has not always been the case. Obviously such apparent irregularities emphasized the necessity for studying the influence of temperature and moisture under controlled conditions, and, accordingly, a series of such experiments was planned and carried out, the results of which are presented herewith.

EXPERIMENTAL METHODS

All of these studies were conducted under greenhouse conditions in the soil-temperature tanks and in controlled-temperature chambers of the Wisconsin Agricultural Experiment Station, at Madison. The soil-temperature apparatus and the general methods employed in these studies were identical with those used in the studies on the *Helminthosporium* disease (12). The temperature chambers have been described by Dickson (4) and it is unnecessary to discuss them in this paper.

The soil used in these studies was a fertile loam obtained from a wood lot near Madison. Although this was virgin soil, it was infested with several grass parasites which attack wheat, and it was necessary to disinfect all of it. Usually the soil was subjected to live steam at about 1 pound pressure for four hours. In other cases it was heated for one hour at 10 to 15 pounds pressure. Both methods gave good results and produced no toxic effect on the plants. One lot of this soil contained somewhat more organic matter than the other, as indicated by their moisture-holding capacities of 67 per cent and 53 per cent, respectively.

The various soil moistures employed in any one experiment were obtained by careful weighings and moisture adjustments before putting the soil in the temperature-tank soil containers. All soil moistures were calculated on the basis of the water-holding capacity of the soil and the moisture content of the soil was maintained as nearly constant as practicable throughout the experiments. The pots were weighed each day and water was added to replace that lost.

The wheat seed used in all of the experiments was of the Goldcoin variety generally known as Junior No. 6. All seed was disinfected in a 1:1,000 solution of mercuric chloride for 10 minutes, then thoroughly washed in running water before planting.

The parasites used for inoculation were obtained from Oregon and New York. The Oregon strain was isolated by Hurley Fellows from wheat collected near Corvallis, Oreg., in 1921, by A. G. Johnson, H. P. Barss, and M. B. McKay. The New York strain was kindly supplied by R. S. Kirby. Both strains originated from single ascospores, and they maintained their pathogenicity throughout the work. The virulence of the Oregon strain seemed to become reduced toward the end of the studies, and the New York strain was used in its place. The loss of virulence of the Oregon strain may have been due to the fact that it never sporulated, and consequently all in-

creases had to be made from mycelium." The New York strain produced ascospores rather abundantly at times and these were used for making new cultures.

The parasites were cultured in Erlenmeyer flasks, on a mixture of equal portions of barley and oats which had been thoroughly cooked and sterilized. The fungus increased rapidly on this medium, and the medium did not produce a toxic effect on the wheat plants when it was added to the soil, as was the case when cooked wheat kernels were used. Inoculations were made by adding to the sterilized soil a given weight of the barley-oat medium on which the fungus was growing. This was accomplished by first removing the upper 3 inches of soil from the containers and thoroughly mixing the inoculum with it. The inoculated soil was then returned to the pots and the seed was sown immediately. An amount of uninoculated medium equivalent to the amount added to the inoculated series was added to the controls, and they always were prepared and sown before preparing and planting the inoculated series in order to prevent accidental contamination.

The relative influences of the different temperatures and moistures were determined on the basis of the amounts of disease produced. The method used for determining the amount of disease was essentially the same as that used in the studies on the *Helminthosporium* disease of wheat (12, p. 199-200). However, owing to the fact that *Ophiobolus graminis* is a more vigorous parasite than *Helminthosporium sativum*, the wheat plants were more severely diseased in the experiments under discussion and it became necessary to classify the injuries in a different manner than was done in the previous studies referred to.

Disease manifestations were divided into three groups or types—(1) leaf and stem blight, (2) infection of tiller bases, and (3) root infection. In order that all data should represent the severity of infection as well as the number of plants infected, the various types of injury were given the numerical ratings shown in Table I. Instead of expressing the total amount of disease occurring at a given temperature as a single infection rating, the different types of injury were kept separate and the infection ratings were calculated for each independently. These calculations were made according to the method employed in the studies on the *Helminthosporium* disease previously referred to (12, p. 200).

TABLE I.—Method of determining the numerical ratings for healthy and diseased wheat plants

Type of injury	Class	Degree and type of injury	Numerical rating
Leaf and stem blight.....	1	None.....	0
	2	Leaves yellow.....	1
	3	Plant killed.....	2
Infection of tiller-bases.....	1	None.....	0
	2	Slight.....	1
	3	Moderate.....	2
Root infection.....	4	Abundant.....	3
	1	None.....	0
	2	Slight.....	1
	3	Moderate.....	2
	4	Abundant.....	3

RESULTS

HOST DEVELOPMENT

The best germination of Goldcoin wheat was obtained at soil temperatures ranging from 12° to 20° C. and at soil moistures ranging from 54.4 to 80 per cent of the moisture-holding capacity. As in the case of Harvest Queen and Marquis wheats (12), germination takes place first at the high temperatures and it is retarded at the low temperatures. During the periods of the experiments (20 to 31 days) the plants produced the greatest dry weight of the aboveground portions at temperatures ranging from 20° to 28° C. and at soil moistures ranging from 71.6 to 80 per cent. Root development seemed to be stimulated at soil temperatures ranging from 16° to 26°. All of the soil moistures employed in these experiments seemed to be about equally favorable for root development.

DEVELOPMENT OF THE PARASITE

One of the writers (Davis) has studied the influence of temperature on the growth of the parasite in pure culture, and the results are given in another paper (1). It was found that growth took place at temperatures ranging from 4° to 33° C. The optimum temperatures for growth varied somewhat for the strains of the parasite studied. However, this variation was within the limits of 19° to 23°. The optimum for the New York strain was rather wide, growth seeming to proceed equally well anywhere between 19° and 24°, whereas the optimum temperature for the growth of the Oregon strain of the parasite seemed to be between 23° and 24°.

DISEASE DEVELOPMENT

In no case did *Ophiobolus* infection occur in any of the plants growing in the uninoculated controls (pl. 1, A). The results obtained in all of the inoculated experiments⁴ are tabulated in Tables II, III, IV, V, VI, and VII, and the data are shown graphically in Figures 1 to 7, inclusive. Plate 1, B illustrates the results obtained on a representative one-tenth of the plants grown in the inoculated soil in Experiment 1B.

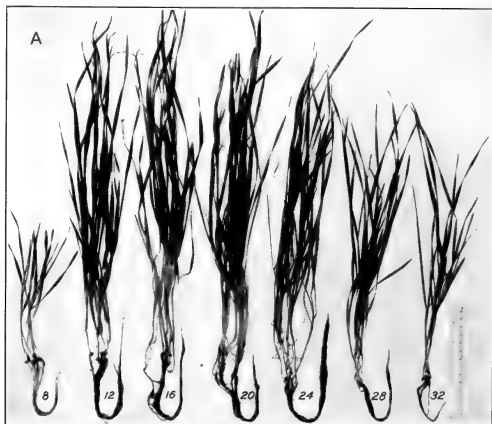
⁴ In MCKINNEY, H. H. TAKE-ALL AND FOOT-ROT INVESTIGATIONS. U. S. Dept. Agr., Bur. Plant Indus., Cereal Courier 14: 23-25. 1922. [Mimeographed] the writers reported a preliminary experiment in which the greatest amount of injury took place in soil held at 22° to 24° C. The population of this experiment was very small and the amount of parasite used was too great for accurate results, as shown by later experiments. Owing to these circumstances and the fact that data were based entirely on top injury, these results are not considered significant and are not included among the results of the subsequent experiments.

EXPLANATORY LEGEND FOR PLATE 1

Influence of soil temperature on the infection of Goldcoin wheat seedlings and young plants by *Ophiobolus graminis*

A.—A representative tenth of all the plants grown at the various soil temperatures in the uninoculated soil of experiment 1B. These plants were all free from infection, as was the case with all of the other uninoculated plants grown in all of the experiments

B.—A representative tenth of all the plants grown at the various soil temperatures in the inoculated soil of experiment 1B. Note the very severe infection in the plants grown at 12° C.; most of these plants died. Also note the severe root rotting on plants grown at soil temperatures below 24° C.



(For explanatory legend see p. 830)

TABLE II.—Effects of soil temperature on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

[These experiments were conducted independently of those in which soil temperature and soil moisture were varied simultaneously]

Experiment 1A ^a					Experiment 1B ^b					Summary ^c			
Average soil temperature, °C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, °C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, °C.	Leaf-blight rating	Tiller-base infection rating	Root-infection rating
8	26	0.0	0.6	33.0	8	31	1.6	13.9	25.2	8	0.8	7.2	29.1
12	26	2.3	32.0	45.5	12	29	30.4	42.5	45.9	12	19.3	37.2	45.7
16	26	3.2	28.2	43.5	16	23	17.3	42.0	42.0	16	10.2	35.1	42.7
20	32	.0	12.5	26.0	20	31	2.2	34.9	40.3	20	1.1	23.7	33.1
24	26	.0	4.5	7.7	24	28	.0	5.9	9.5	24	.0	5.2	8.6
28	26	.0	1.9	3.8	28	21	.0	7.1	3.1	28	.0	4.5	3.4
32	13	.0	3.8	.0	32	18	.0	2.8	4.6	32	.0	3.3	2.3

^a Plants grown in soil having a moisture-holding capacity of 67 per cent and containing 56.1 per cent moisture; one 6-inch soil can used for each temperature; 35 seeds and 100 grams of inoculum containing the Oregon strain of the parasite used in each soil can; started Feb. 14, 1922; ended Mar. 10, 1922.

^b Experiment carried on at the same time and in the same manner as experiment 1A, except that it was not ended until Mar. 17, 1922.

^c Average amount of infection at each soil temperature in experiments 1A and 1B.

TABLE III.—Effects of soil temperature on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

[These results are from experiments which were a part of the soil-moisture experiments and comprise all of the data obtained in soil of relatively low-water content]

Experiment 2A ^a					Experiment 3A ^b					Experiment 4A ^c					Summary ^d			
Average soil temperature, °C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, °C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, °C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, °C.	Leaf-blight rating	Tiller-base infection rating	Root-infection rating
8	34	2.9	10.7	75.9	8	39	0.0	35.9	56.0	8	48	0.0	14.5	38.8	8	0.9	20.3	56.9
12	26	42.3	79.4	84.0	12	46	.0	65.2	49.0	12	46	23.8	55.8	80.0	12	22.0	66.8	71.0
16	29	24.4	78.1	69.5	16	54	16.6	69.7	68.0	16	50	28.0	78.6	81.9	16	23.0	75.4	73.1
20	24	22.9	44.4	69.5	20	53	2.8	41.5	38.0	20	50	7.0	67.3	70.6	20	10.9	51.1	59.4
24	12	.0	2.8	5.5	24	53	2.8	40.8	47.0	24	51	9.8	38.5	62.7	24	4.2	27.3	38.4
28	20	5.0	.0	.0	28	45	.0	6.6	.0	28	43	.0	10.0	16.6	28	1.6	5.5	5.5

^a Plants grown in soil having a moisture-holding capacity of 67 per cent and containing 33.8 per cent moisture; one 6-inch soil can used for each temperature; 35 seeds and 100 grams of inoculum containing the Oregon strain of the parasite used in each soil can; started June 3, 1922; ended July 1, 1922.

^b Plants grown in soil having a moisture-holding capacity of 53 per cent and containing 33.2 per cent moisture; one 8-inch soil can used for each temperature; 60 seeds and 400 grams of inoculum containing the Oregon strain of the parasite used in each soil can; started Feb. 21, 1923; ended Mar. 14, 1923.

^c Plants grown in soil having a moisture-holding capacity of 53 per cent and containing 33.2 per cent moisture; one 8-inch soil can used for each temperature; 60 seeds and 200 grams of inoculum containing the New York strain of the parasite used in each soil can; started Mar. 31, 1923; ended Apr. 20, 1923.

^d Average amount of infection at each soil temperature in experiments 2A, 3A, and 4A, having soil moistures of 33.8, 33.2 and 33.2 per cent, respectively.

TABLE IV.—Effects of soil temperature on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

[These results are from experiments which were a part of the soil moisture experiments and comprise all of the data obtained in soil of medium water content]

Experiment 2B ^a					Experiment 3B ^b					Experiment 4B ^c					Summary ^d			
Average soil tem- perature, °C.	Number of plants	Leaf-blight rating	Tiller-base infec- tion rating	Root-infection rating	Average soil tem- perature, °C.	Number of plants	Leaf-blight rating	Tiller-base infec- tion rating	Root-infection rating	Average soil tem- perature, °C.	Number of plants	Leaf-blight rating	Tiller-base infec- tion rating	Root-infection rating	Average soil tem- perature, °C.	Leaf-blight rating	Tiller-base infec- tion rating	Root-infection rating
8	32	0.0	1.0	9.4	8	54	0.0	63.5	64.7	8	49	0.0	33.3	33.3	8	0.0	32.6	35.8
12	33	1.5	64.6	44.7	12	54	.0	69.1	70.0	12	49	70.4	94.5	98.5	12	23.9	76.0	71.0
16	31	11.2	18.2	10.7	16	51	36.2	97.4	76.4	16	57	72.8	94.7	99.5	16	40.0	70.1	62.2
20	27	16.6	3.7	12.3	20	42	30.9	92.0	79.0	20	51	71.5	94.8	93.1	20	39.7	63.5	61.4
24	20	.0	.0	.0	24	44	28.4	78.7	70.0	24	49	60.2	93.1	89.3	24	29.5	57.3	53.1
28	17	.0	1.9	.0	28	47	2.1	26.2	37.0	28	50	.0	64.0	66.0	28	.7	30.7	34.3
32	5	.0	.0	.0											32	.0	.0	.0

^a This experiment was carried on at the same time and in the same manner as experiment 2A, except that the soil moisture was held near 54.4 per cent.

^b This experiment was carried on at the same time and in the same manner as experiment 3A, except that the soil moisture was held near 55 per cent.

^c This experiment was carried on at the same time and in the same manner as experiment 4A, except that the soil moisture was held near 56 per cent.

^d Average amount of infection at each soil temperature in experiments 2B, 3B, and 4B having soil moistures of 54.4, 55, and 56 per cent, respectively.

TABLE V.—Effects of soil temperature on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

[These results are from experiments which were a part of the soil moisture experiments and comprise all of the data obtained in soil of relatively high water content]

Experiment 2C ^a					Experiment 3C ^b					Experiment 4C ^c					Summary ^d			
Average soil temperature, ° C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, ° C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, ° C.	Number of plants	Leaf-blight rating	Tiller-base infection rating	Root-infection rating	Average soil temperature, ° C.	Leaf-blight rating	Tiller-base infection rating	Root-infection rating
8	35	0.0	12.3	66.6	8	45	0.0	33.3	66.6	8	57	0.0	29.2	33.3	8	0.0	24.9	55.5
12	26	28.8	62.8	73.3	12	46	15.2	67.4	75.2	12	48	53.1	65.9	88.3	12	32.3	65.3	78.9
16	28	32.1	77.3	81.0	16	43	79.0	97.6	98.3	16	60	72.5	85.0	95.5	16	61.2	86.6	91.6
20	20	42.5	58.3	79.0	20	56	36.6	93.4	84.5	20	46	66.3	89.8	88.3	20	48.4	80.5	83.7
24	16	.0	18.7	43.7	24	37	67.5	94.5	96.4	24	48	76.0	88.2	96.8	24	47.8	67.1	78.9
28	19	.0	15.8	20.3	28	32	40.6	87.5	90.4	28	27	7.4	37.0	37.6	28	16.0	46.7	49.4
32	5	.0	6.6	20.0	32	---	---	---	---	32	---	---	---	---	32	.0	6.6	20.0

^a This experiment was carried on at the same time and in the same manner as experiment 2A, except that the soil moisture was held near 71.6 per cent.

^b This experiment was carried on at the same time and in the same manner as experiment 3A, except that the soil moisture was held near 80 per cent.

^c This experiment was carried on at the same time and in the same manner as experiment 4A, except that the soil moisture was held near 80 per cent.

^d Average amount of infection at each soil temperature in experiments 2C, 3C, and 4C having soil moistures of 71.6, 80, and 80 per cent, respectively.

TABLE VI.—Effects of variations in soil and air temperature on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

[The plants were grown in large chambers in which both temperature and humidity were controlled within relatively close limits. The soil was inoculated with the New York strain of the parasite, and 15 disinfected seeds were planted in each of six containers for each temperature chamber. Experiment started March 31, 1923, and ended April 27, 1923]

Temperature range, ° C.	Number of plants	Tiller-base infection rating	Root-infection rating
9.5 to 13.2.....	70	76.3	97.9
15.8 to 17.0.....	68	85.1	96.4
26.5 to 27.5.....	59	21.4	36.1

TABLE VII.—Summary of data given in Tables III, IV, and V, arranged to show the influence of soil moisture on the infection of young Goldcoin wheat plants with *Ophiobolus graminis*

Leaf blight				Tiller-base infection				Root infection			
Average soil temperature, ° C.	Low soil moisture	Medium soil moisture	High soil moisture	Average soil temperature, ° C.	Low soil moisture	Medium soil moisture	High soil moisture	Average soil temperature, ° C.	Low soil moisture	Medium soil moisture	High soil moisture
8	0.9	0.0	0.0	8	20.3	32.6	24.9	8	56.9	35.8	55.5
12	22.9	23.9	32.3	12	66.8	76.0	65.3	12	71.0	71.0	78.9
16	23.0	40.0	61.2	16	75.4	70.1	86.6	16	73.1	62.2	91.6
20	10.9	39.7	48.4	20	51.1	63.5	80.5	20	59.4	61.4	83.7
24	4.2	29.5	47.8	24	27.3	57.3	67.1	24	38.4	53.1	78.9
28	1.6	.7	16.0	28	5.5	30.7	46.7	28	5.5	34.3	49.4
32	-----	0	.0	32	-----	.0	6.6	32	-----	.0	20.0

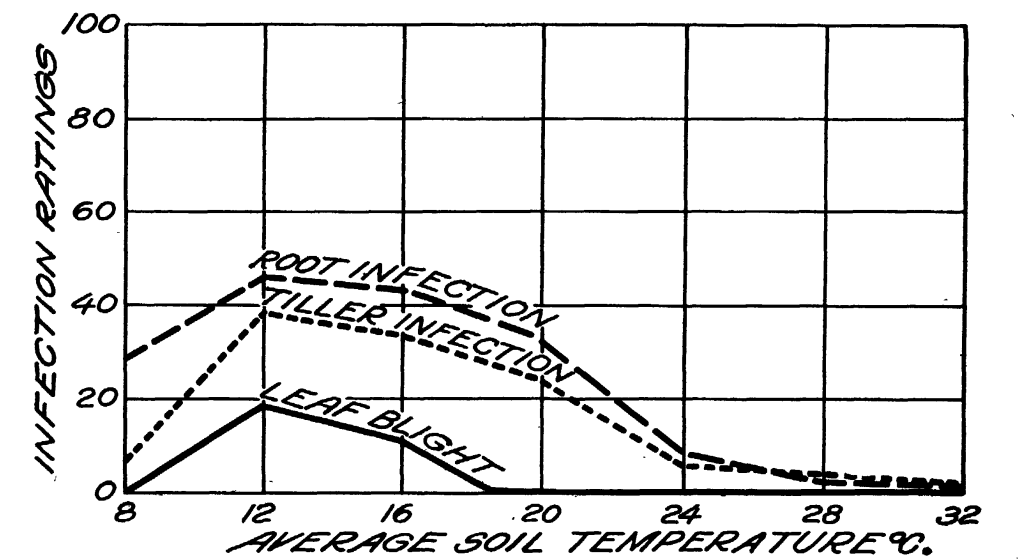


FIG. 1.—Curves showing summaries of infection ratings on the roots, tiller bases, and aboveground parts of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown in soil containing 56.1 per cent of moisture, and held at different temperatures, as shown in Table II

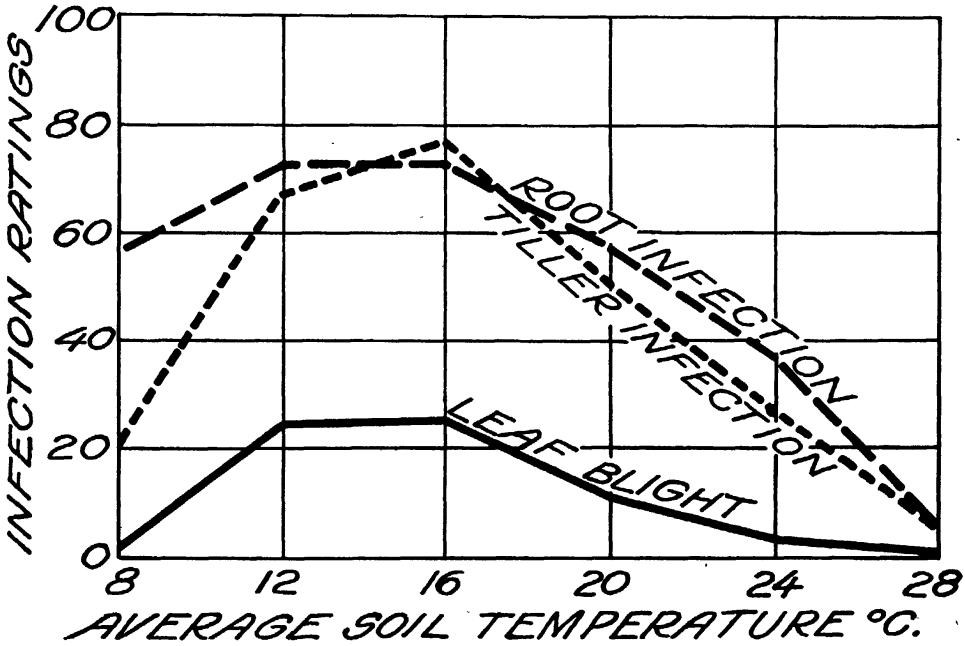


FIG. 2.—Curves showing summaries of infection ratings on the roots, tiller bases, and aboveground parts of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown in soil containing from 33.2 to 33.8 per cent of moisture, and held at different temperatures, as shown in Table III

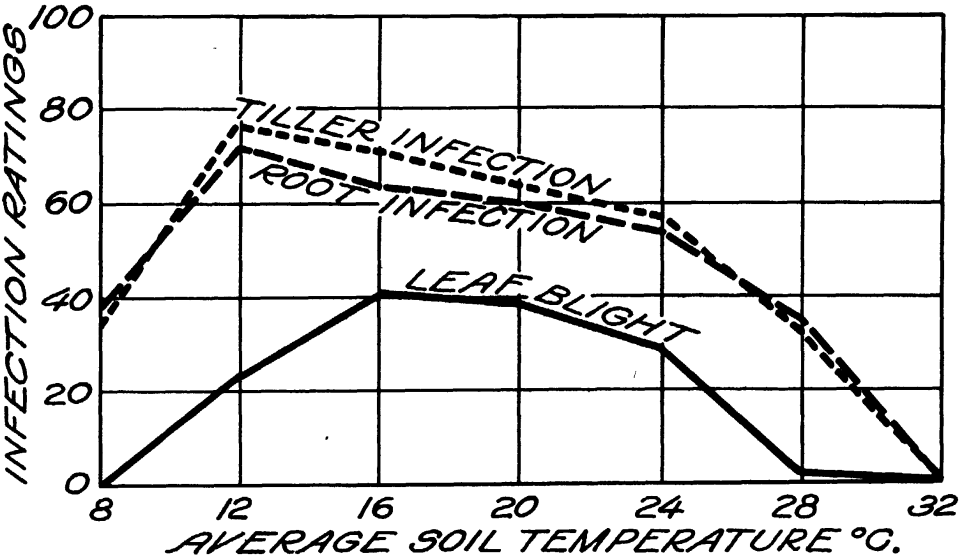


FIG. 3.—Curves showing summaries of infection ratings on the roots, tiller bases, and aboveground parts of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown in soil containing from 54.4 to 56 per cent of moisture, and held at different temperatures, as shown in Table IV

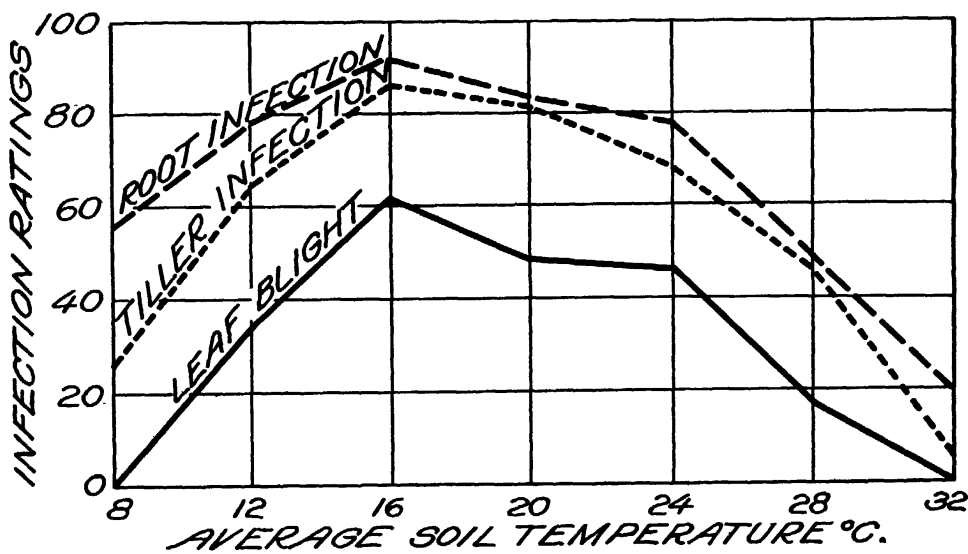


FIG. 4.—Curves showing summaries of infection ratings on the roots, tiller bases, and aboveground parts of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown in soil containing from 71.6 to 80 per cent of moisture, and held at different temperatures, as shown in Table V

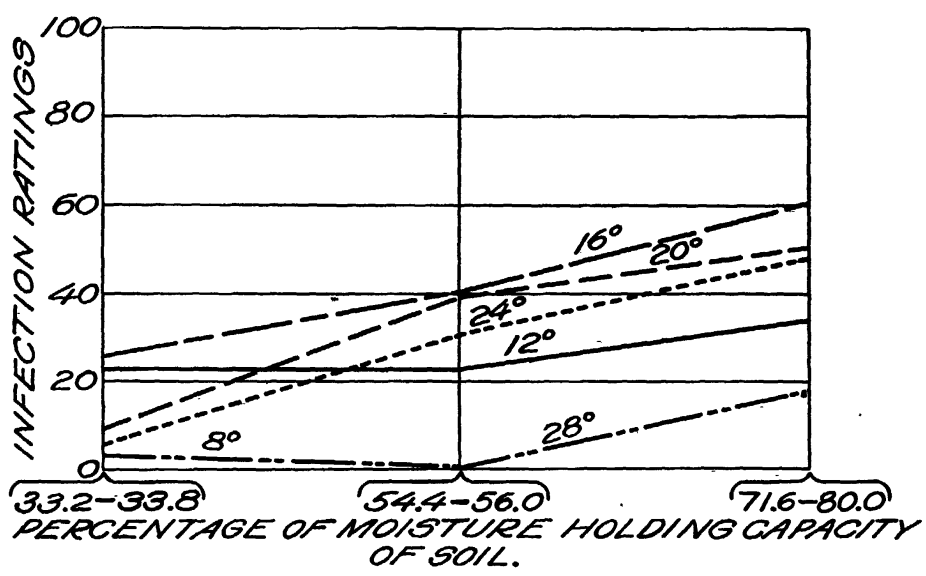


FIG. 5.—Curves showing summaries of infection ratings on the aboveground parts of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown at different soil moistures when soil temperature was varied simultaneously. Tabular results are given in Table VII

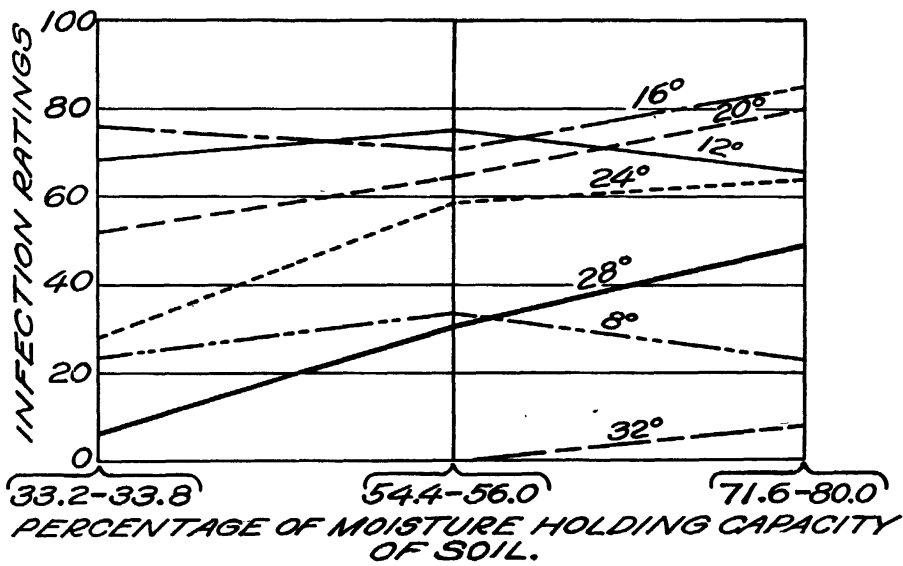


FIG. 6.—Curves showing summaries of infection ratings on the tiller bases of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown at different soil moistures when soil temperature was varied simultaneously. Tabular results are given in Table VII

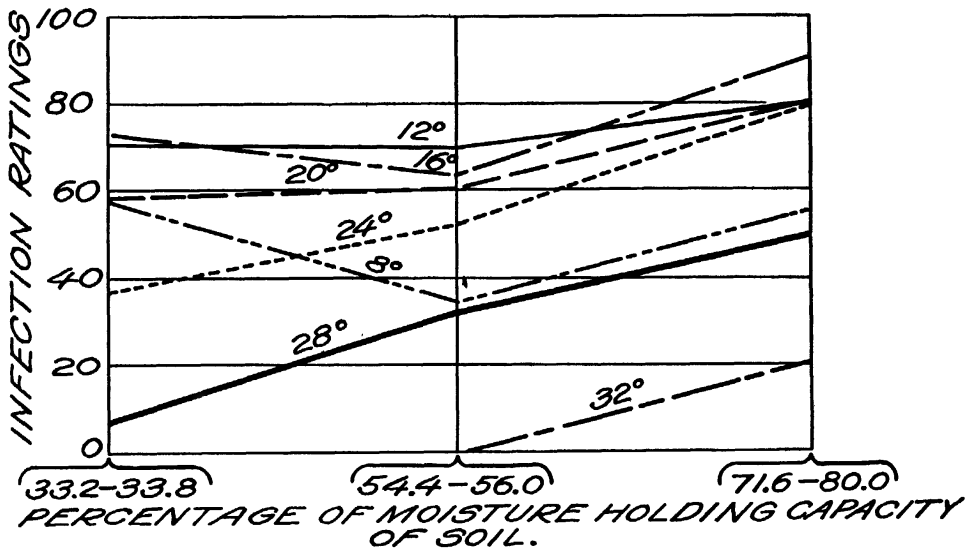


FIG. 7.—Curves showing summaries of infection ratings on the roots of Goldcoin wheat plants inoculated with *Ophiobolus graminis*, when grown at different soil moistures when soil temperature was varied simultaneously. Tabular results are given in Table VII

From the data presented, it is evident that *Ophiobolus graminis* is a vigorous root and tiller-base parasite, and also that infection is greatly influenced by soil temperature and soil moisture. Although the temperature and moisture optima shifted slightly in the several experiments, it is evident that infection and injury are favored by moderately low temperatures (12° to 16° C.) and by fairly high soil moistures (70 to 80 per cent). In these studies it was found that *O. graminis* causes practically no injury to the very young seedlings just before emergence. The germination of seeds planted in the inoculated soils was not consistently lower than that of seeds sown in the uninoculated soil; in fact it was not uncommon to find that the germination of seed in the control soil was actually lower than that of seeds sown in the inoculated soil. It was found in these studies that infection seldom took place until the seedlings were well advanced. This was found to be the case even in the experiments in which the greatest quantities of inoculum were used. When small quantities of inoculum were used the plants showed little infection until they were well advanced beyond the seedling stage.

An examination of the summary curves in Figures 2, 3, and 4 shows that the optimum temperature for tiller-base and root injury was 12° C. in soil containing the medium amount of water, whereas 16° C. was the optimum temperature for these injuries in soils containing low and high soil moistures. Whether this shift represents an actual relationship between the joint influences of temperature and moisture or merely experimental variation is a question. The data given for the individual experiments show that the temperature optima shifted from time to time, regardless of the moisture content of the soil. This indicates that some other factors which were not so well controlled as soil temperature and soil moisture may have a decided influence on the occurrence of the disease and that the temperature optimum probably extends over a range of at least 4° to 6° . The quantity of *Ophiobolus* inoculum placed in the soil has a marked influence on the behavior of the take-all disease, as was also found in the studies on *Actinomyces scabies* (8) and *Helminthosporium sativum* (12). However, it is much more difficult to standardize the amount of inoculum of *Ophiobolus graminis* than is the case with the inoculum of the other parasites mentioned, and as the strains of *O. graminis* employed in these studies differed in their virulence it was frequently found that the quantity of inoculum used was in excess of that which would give the sharpest indications of the temperature and moisture optima.

DISCUSSION

Although these studies have only opened up the general subject of environmental influence on the development of the take-all disease, it is believed that the data presented represent the general influences of temperature and moisture on the infection of wheat seedlings and young wheat plants by *Ophiobolus graminis*. While it is difficult or impossible to correlate many of the field observations which have been recorded on this subject, it now appears from the experimental data herein presented that some of the seemingly contradictory reports may be in accord with the facts. As pointed out earlier, the quantity of infectious material in the soil influences the amount and severity of take-all, and this relationship doubtless is the basis for many discrepancies in field observations.

In these experiments of the writers it was found that all infected plants did not show signs of the disease on the aboveground parts. In many cases plants which appeared healthy before removal from the soil were in reality almost devoid of a root system. This condition has been noted also by one of the writers (McKinney) in several commercial wheat fields which were affected by take-all. Although data presented here show that relatively high soil moistures favor infection and injury, it is evident that these soil moistures also were favorable for the development of the host. At these soil moistures many plants sent out new roots in an attempt to replace those which had become rotted. In the present studies the writers did not subject any particular lot of plants to various temperature and moisture changes in order to determine the exact influence of hot, dry conditions following a period of cold, moist conditions or vice versa. However, on a basis of our knowledge of plant responses it is only reasonable to assume that even though cool, moist conditions do favor infection and injury, a sudden reduction in water supply and increased transpiration would produce marked leaf yellowing and killing among those apparently healthy plants which in reality have badly rotted culms and roots. One of the writers (McKinney) has noted an increase in the amount of leaf yellowing in infested fields after the beginning of a hot, dry period. Under such conditions, it might be assumed that dry, hot weather actually favors infection, but it appears that such weather only brings out the expression on the aboveground parts of injuries below ground which are favored by the opposite set of conditions. It would seem that the nonappearance, in the fall and extremely early spring, of take-all on winter wheat on infested land is probably accounted for on a basis of cool, moist conditions which favor the growth of the wheat plants although already infected. However, when the warm spring days arrive the infected plants turn yellow and finally die.

Although the optimum temperature for the development of the parasite is more limited than that for the host, it is of interest to note that the two optima appear to be very close together. On the other hand, the optimum temperature range for the disease is considerably lower than that for either the host or parasite. This relationship is exactly the reverse of that existing with the *Helminthosporium* disease of wheat, the latter disease being favored by soil temperatures which are above those favorable for the host and parasite. In a paper dealing with this latter disease, McKinney (12) suggested that the high temperature optimum for disease occurrence might be accounted for on the basis of the weakening of the host at the high, unfavorable temperatures. However, it is not possible to explain the results with *Ophiobolus graminis* on such a basis. At 12° and 16° C. the wheat plant is thrifty and robust, yet it is attacked severely by this parasite. *Ophiobolus graminis* produces severe injury at unusually low temperatures in comparison with the other wheat parasites such as *Gibberella saubinetii*, studied by Dickson (2), and *Helminthosporium sativum*, studied by the senior writer (12). It seems that the explanation of this will require the study of factors relating to both the host and the fungus and which are far more basic than the phenomena of growth rate and vigor as expressed by the ordinary methods of weight and measurement. It is believed that this also applies to the explanation of the behavior

of the Helminthosporium disease. This is supported by the results obtained by Dickson, Eckerson, and Link (3) with two hosts (corn and wheat) which possess different disease-temperature optima when attacked by *G. saubinetii*. It will be especially important to investigate these problems further, not only by the use of additional hosts and one parasite but by employing one host and several parasites which possess different disease-temperature optima. Unquestionably *H. sativum*, *O. graminis*, and the wheat plant offer opportunities in this direction.

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BACTERIAL SPOT OF COWPEA AND LIMA BEAN¹

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INTRODUCTION

The bacterial spot disease of cowpeas was first noted in southern Indiana in 1919, but its bacterial nature was not determined until 1921, when it occurred in an experimental plot of cowpeas at La Fayette, Ind. It is a typical spot disease of the leaves, stems, and pods, distinctly different from the other bacterial diseases of cowpeas reported in the literature. The organism which causes this disease also causes a very similar and widespread spot disease of lima beans which has been described recently by Tisdale and Williamson (23, 24).³ The work herein reported deals mainly with the symptoms of the disease as it occurs on cowpeas, the isolation, characteristics, overwintering, pathogenicity, and dissemination of the causative bacteria, and the identity of the latter with the species causing the lima-bean disease.

HISTORY AND OCCURRENCE

A search of the literature has not revealed any previous description of this disease of cowpeas, although the symptoms of the spot disease of lima beans and cowpeas described by Smith (18, p. 15) in 1905, and attributed to *Phyllosticta phaseolina* Sacc., closely resemble those of the disease under consideration. A number of cases of bacterial infection of cowpeas have been recorded. Smith and McCulloch (19), in 1919, reported a wilt of cowpeas produced by inoculation with *Bacterium solanacearum* E. F. S. Smith (20, p. 280) mentions a bacterial spot of cowpeas, but does not describe the disease. Rapd (15, p. 3) in 1920 and more recently Burkholder (3, p. 7) have reported infection of cowpeas with *Bacterium phaseoli* E. F. S., an organism which, however, differs radically in culture from the organism causing the spot disease. Wolf and Foster (25, p. 452) have isolated the tobacco wildfire organism (*Bact. tabacum* Wolf and Foster) from small, yellowish leaf spots on cowpeas grown near infected tobacco, but attribute the cowpea lesions to infection about leafhopper wounds. Osmun (14) has isolated the wildfire organism from lima beans grown adjacent to tobacco, and Johnson, Slagg, and Murwin (10, p. 177) successfully inoculated cowpeas with the organism. However, this organism differs culturally from the one causing the cowpea spot disease, and the writers' attempts to inoculate tobacco have failed. The history of bacterial spot as it occurs on lima bean has been given by Tisdale and Williamson (24). Chupp has reported to the Federal Plant Disease Survey the presence since 1918 of the disease on lima beans in New York.

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² The writers wish to acknowledge their indebtedness to Prof. H. S. Jackson for his advice and criticism.

³ Reference is made by number (italic) to "Literature cited," p. 862.

The disease on cowpeas was first found at Vallonia, Ind., in August, 1919, and was next found at Decker, in southern Indiana, in August, 1920. In August, 1921, it occurred in an experimental plot of Whip-poorwill cowpeas at La Fayette, Ind., and has occurred in the writers' plots in 1922, 1923, and 1924. The disease appears to be very widespread, since it has been found in seed from South Carolina, Virginia, and Washington, D. C., and was noted in the field in 1922 near Decker, Ind., Seaford, Del., and Louisville, Ky. Seiyo Ito, who examined the writers' plots at La Fayette, said that the same disease also occurs in Japan.

In 1923 the disease was found in Knox County, Ind., and in Kansas by R. P. White, and in Florida by W. B. Tisdale, who also reported it as a serious trouble in 1924. In 1924 the disease was serious in the field crop (variety, New Era) in Jackson County, Ind., and was more severe in the writers' plots at La Fayette than in the preceding three years. This disease as it occurs on lima beans was noted commonly in gardens in Indiana in 1919 and 1920, and it occurred to a considerable extent in 1923 and 1924.

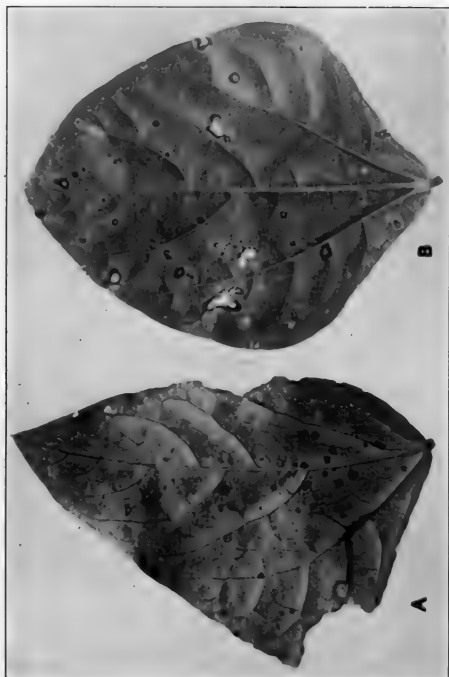
HOSTS

Greenhouse inoculations and field studies have shown that the following are hosts of this parasite: Cowpea (*Vigna sinensis* [L.] Endl.); catjang (*Vigna catjang* Walp.); hyacinth bean (*Dolichos lablab* L.); Florida velvet bean (*Stizolobium deeringianum* Bort); adsuki bean (*Phaseolus angularis* Wight); lima bean (*Phaseolus limensis* Macf.), as represented by the Large White Pole, Giant Podded Pole, and King of the Garden varieties; bush lima bean (*Phaseolus limensis* Macf. var. *limenanus* Bailey), as represented by the Burpee's Bush and Fordhook varieties; and the dwarf sieva bean (*Phaseolus lunatus* L. var. *lunonanus* Bailey), as represented by the Henderson's Bush variety. Tisdale and Williamson (24) had found the varieties Burpee's Bush, Fordhook, King of the Garden, Dreer's Bush, and Henderson's Bush to be susceptible. Natural infection with what is in all probability the same organism was found rather abundantly on the leaves of the common native weed, tick trefoil (*Desmodium canescens* [L.] DC.), in a fallow field near La Fayette, Ind., on August 23, 1924, and has also been found on asparagus bean (*Vigna sesquipedalis* Wight) in field plots.

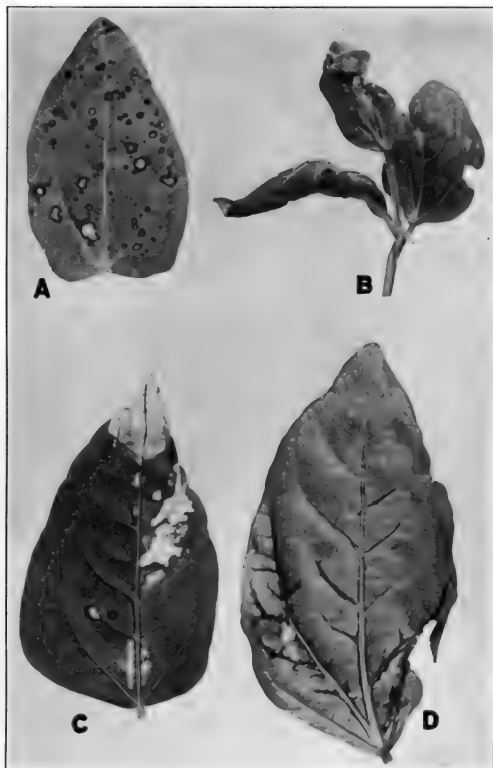
All of the following 23 varieties of cowpeas tested have proved to be susceptible: Whip-poorwill, Brabham, Early Red, Early Black, Early Buff, Taylor, Black, Red Ripper, Iron, Conch, Groit, New Era, Clay, Wonderful, California Blackeye, Early Ramshorn Blackeye, Cream Chowder, Gallavant, Large Blackeye, Arlington, Columbia, Progressive White, and Victor.

SYMPTOMS

On the cowpea leaves, the spots as usually seen in the field are irregularly circular or lobed rather than angular, and are 1 to 4 mm. broad, sometimes larger. Very young spots are small, circular, sunken dots, first water soaked or greasy, later claret-brown in color (16) (pl. 1, A). The larger lesions are characterized by a buff center surrounded by a conspicuous maroon or claret-brown margin about 1 mm. in width (pl. 1, B; pl. 2, A). Leaf lesions frequently become



A.—Lower surface of cowpea leaflet, showing small sunken lesions; and, on lower left side, a large lesion extending along a vein. $\times 2$
B.—Upper surface of cowpea leaflet, showing lesions, older than those in A, with light centers and reddish-brown margins. In some the centers have fallen out. The large lesion near left-hand edge has an extension along a vein. $\times 2$



A.—Catjang leaflet, showing bacterial spot lesions
 B.—Cowpea leaf. Infected early along the veins. Note distortion
 C.—Cowpea leaflet. Infected early. Note bleached areas
 D.—Lower side of cowpea leaflet, showing invasion along the vein

rather large, bleached, dried areas surrounded by a claret-brown border (pl. 3, B). In old lesions the central tissue dries and may crack or drop out. Frequently the central tissue is invaded by fungi.

The lesions are not in any way delimited by the veins; on the contrary they frequently extend markedly along the veins (pl. 2, D; pl. 4, D and E). Linear leaf lesions have been noted extending along a vein a distance of 4 to 6 cm. If young leaf lesions are mounted in a drop of water on a slide and cut across with a scalpel, the bacteria may readily be seen under the microscope, oozing out in cloudy masses from the cut edge, especially from the region of the veins.

Infection occurs very readily on young growing leaves, and considerable distortion, curling, tearing, and puncturing of the leaves may be caused by the growth stresses (pl. 2, B; pl. 3, B). Lesions of early inception may thus become maroon-bordered slits and tears in the leaf lamina or along its margin, and vein lesions may cause curvature and crinkling. Lesions on a vein may cause a yellowing or bleaching (pl. 2, C) of distal portions of the leaf.

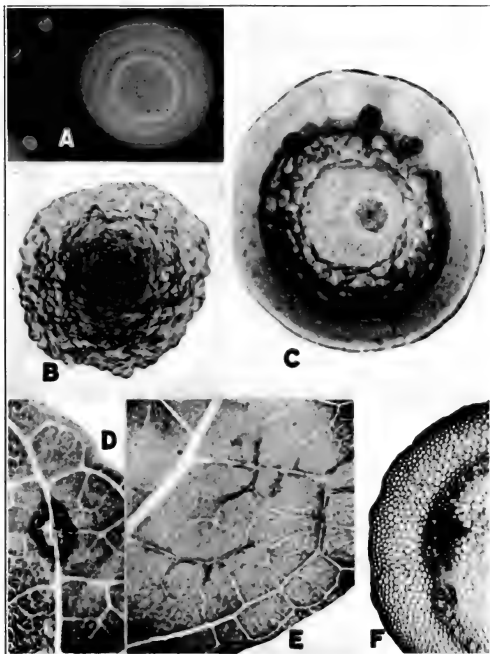
Lesions on the young leaves of cowpea seedlings under greenhouse conditions first become visible as small sunken pits or craters in the epidermis (pl. 5, C), which are translucent in transmitted light but soon become greasy or water-soaked and later claret-brown in color. At first such lesions are visible only on the lower side of the leaf and as a rule remain more extensive and more conspicuous there than on the upper side of the leaf. Older lesions assume the buff center and the claret-brown border and are surrounded by a narrow light-green halo. Also very small claret-brown lesions occur on the stipules (pl. 6, D). The symptoms on lima-bean leaves, described by Tisdale and Williamson (24), are essentially similar to those on cowpea leaves, as are also the lesions on catjang leaves (pl. 2, A). On the leaves of artificially infected velvet-bean plants the lesions were dark brown to black, with some tendency to be delimited by the veins (pl. 6, F). Natural infection of velvet-bean leaves produced lesions more closely resembling those on cowpea. These were irregularly circular, with a tan center and a dark-brown marginal line, and were surrounded by a light-green or yellowish halo in the living tissue. On tick trefoil the lesions showed a tan to light-brown center, with a narrow, reddish-brown marginal line, and were surrounded by a light-green or yellowish halo in the living tissue (pl. 5, B).

On the cowpea stems and petioles the lesions are more or less oval, 1 to 5 mm. long, and Victoria lake in color (pl. 6, D and E). The center is usually sunken, and there may be water-soaked tissue above and below the lesion. Frequently the lesions are much longer, especially on the petioles, and large sunken lesions are formed on the epicotyls and hypocotyls of seedlings (pl. 3, F and G). Similar stem and petiole infection also occurs on lima beans. Catjang leaves have been noticed to break off at petiole lesions.

The lesions on cowpea and catjang pods are irregularly circular and 1 to 8 mm. in diameter, and morocco red, claret brown, maroon, or Victoria lake in color (pl. 3, A). The larger lesions often have a sunken center and a water-soaked outer border. Infection of young pods results in a marked constriction of the pod at the point of infection, and usually in an abnormal bending of the pod at that



- A.—Full-grown cowpea pod, showing lesions which have not caused conspicuous constrictions
- B.—Cowpea leaflet, showing large lesions with tan centers and reddish brown borders. Such lesions result from early infection and cause malformation and shattering of leaves
- C.—Normal cowpea seed above. Two infected, shriveled, discolored seeds below, for comparison. $\times 2$
- D.—Seedling from an infected seed, showing the cotyledon lesion. $\times 2$
- E.—Portion of cowpea pod, showing the large normal seeds in the healthy part of the pod and the small, dark, infected seeds under a bacterial-spot lesion. $\times 2$
- F.—Portion of seedling stem, showing a large lesion extending down the hypocotyl from the point of attachment of the cotyledon. Such a lesion is the result of invasion from a cotyledon lesion such as is shown in D. $\times 2$
- G.—Hypocotyl of seedling enlarged ($\times 2$) to show sunken lesions



A.—Agar-plate surface colony, with smaller submerged colonies at left. $\times 5$
 B.—Surface colony on a very dry agar, showing lobed margin and surface sculpturing. $\times 20$
 C.—Surface colony such as that shown in A, showing internal pattern. $\times 17$
 D.—Cowpea leaf lesion, showing that the veins do not limit the spread of the invasion. $\times 9$
 E.—Infection extending along the veinlets in a cowpea leaf. $\times 9$
 F.—Cross section of hypocotyl of cowpea, showing bacterial invasion of xylem elements as a result of infection proceeding from a cotyledon lesion. $\times 34$

point (pl. 6, A, B, and C). Sometimes the entire distal portion of the pod fails to enlarge. Large lesions frequently penetrate through the ovary wall to the seed, causing a stunting, shriveling, and dark discoloration of the latter (pl. 3, C and E). However, attempts to separate upon this basis the infected from the healthy in commercial seed, have been unsuccessful. Tisdale and Williamson (24) found similar pod lesions and resultant seed infection in the lima bean.

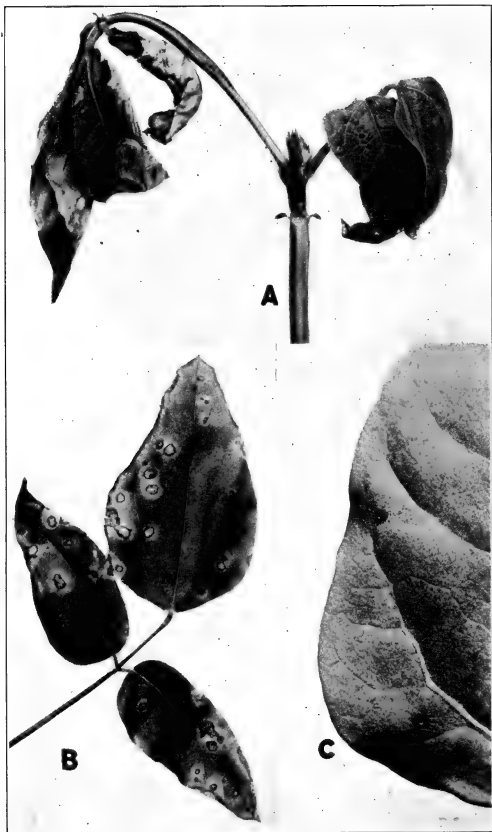
In cowpea seedlings from infected seed the cotyledons bear large lesions which frequently cause a water-soaking or dark discoloration and a shriveling or constriction of a considerable portion of the cotyledon (pl. 3, D). Such lesions are frequently accompanied by a transverse crack or fissure, and may kill the cotyledon prematurely. Oval, maroon lesions also occur on the hypocotyls and epicotyls of such seedlings (pl. 3, F and G).

From the infected cotyledons or from epicotyl lesions, infection of the vascular bundles occurs (pl. 4, F), and the invasion frequently extends up along one or more bundles through the petiole and out into the veins of the first leaf. This type of infection is visible as an internal reddish-brown streak within the vascular bundles, and causes a yellowing or wilting and blighting of all or portions of one or both of the first leaves. The affected portions of such leaves may show a darkened network of veins (pl. 2, C), and such infection has been observed to cause a rather extensive, shiny, brownish discoloration of the lower surface of the leaf along the veins. In some cases a preliminary yellowing of the veinlets and a stunting of the corresponding half of each leaf was observed. In extreme cases the entire seedling may be stunted or may wilt and die. Similar wilting and blighting effects have been noted on the first compound leaves of fieldgrown seedlings (pl. 5, A), and also on older plants as a consequence of early infection of the petiole which had resulted in extensive internal invasion of the vascular tissues. Under such conditions, irregular bleached areas of considerable extent frequently occurred on the leaf blades (pl. 2, C), accompanied sometimes by an extensive reddish discoloration of the lower epidermis.

The tendency to invade the veins has been noticed in the case of lesions on or near the veins of young leaves, in which case a brown streak may be traced out through or along the vein and into its smaller branches (pl. 1, A, B; pl. 2, D). Such infection may cause a yellowing or wilting of portions of the lamina supplied by the infected vein. Thus, although this is typically a spot disease, there are distinct evidences of localized vascular infection, particularly in the seedlings, which results in symptoms of a systemic nature.

In the Early Buff variety of cowpea this disease should not be confused with another spot disease with which a species of *Cladosporium* is associated. The latter trouble is characterized by smaller purple to black spots on the pods, irregular in outline, and by oval, sunken, purplish spots on the young stems and peduncles. These lesions may have a tan center on which appears a greenish, velvety, fungous growth. Pod infection occurs very early and often causes great distortion of the pods.

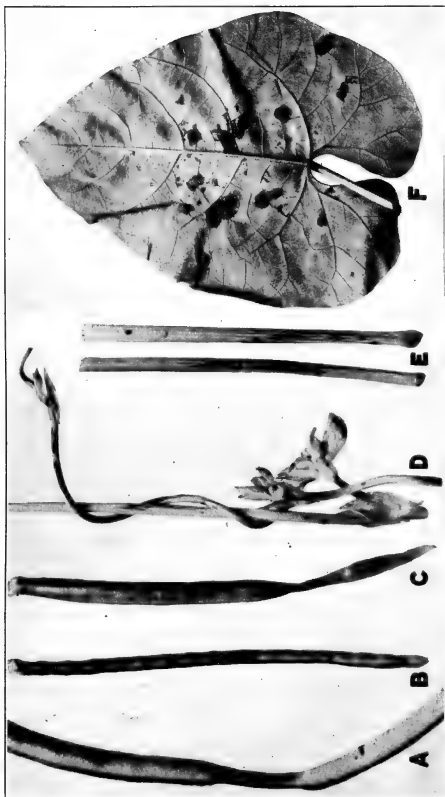
Early stages of the leaf spot caused by *Amerosporium oeconomicum* E. and T., as observed on the New Era variety, somewhat resemble bacterial spot, but the white center, concentric rings, and pycnidia of older lesions serve as distinguishing characters. The



A.—Wilting of leaves of field-grown cowpea seedling as a result of vascular infection in the stem and petiole

B.—Lesions on leaf of tick trefoil resulting from natural infection. The dry centers are tan or light brown, surrounded by a narrow dark-brown margin and a light-green or yellow halo

C.—Craterlike lesions on upper side of a first leaf of a cowpea seedling as a result of seed-borne infection $\times 3$



A.—Portion of immature cowpea pod, showing a constriction due to two lesions resulting from early infection.
 B.—Young cowpea pod, showing stage at which growth is arrested by infection which results in such constrictions as are seen in A and C.
 C.—Young cowpea pod failing to enlarge at the infected point.
 D.—Lesions on stems and bracts of cowpea.
 E.—Lesions on petioles of cowpea.
 F.—Lesions on velvet bean leaflet resulting from atomizer inoculation.

early stages of the leaf lesions caused by *Cercospora cruenta* Sacc. are more diffuse and less clearly defined. The older lesions are larger than the bacterial lesions, with a darker center and less conspicuous border than the latter, and show some tendency, at least in the Blackeye variety, to be delimited by the larger veins. The abundant sporulation of the fungus is of course a reliable differential characteristic.

ECONOMIC IMPORTANCE

The bacterial spot disease may cause very severe foliage injury to cowpea seedlings and young plants, especially in wet seasons, and not only kills leaves but may even cause the death of many young plants. Tisdale has reported through the Federal plant disease survey that this disease caused a serious defoliation of cowpeas in Florida. In the cowpea crop grown for seed the pod lesions of this disease may cause considerable loss. In the case of lesions constricting and stunting the pod, the number of seeds is reduced, and seeds borne under lesions are stunted or shriveled, are impaired in germinability, and produce diseased and weakened seedlings. Leaf and stem infection on older plants is, as a rule, less destructive. The disease seems to be very destructive to lima beans, especially in its leaf attack (24).

CAUSAL ORGANISM

ISOLATION

The bacterial nature of the disease on cowpeas was discovered in the late summer of 1921. On August 26 the surface of a spotted pod was sterilized in a 1:1,000 solution of mercuric chloride and a lesion was cut out with a flamed scalpel and macerated in a drop of sterile water on a flamed slide. This drop of water was plated out in potato-dextrose agar by the loop-dilution method, and after three days at room temperature the plates were evenly seeded with similar grayish-white bacterial colonies. The surface was sliced off from another pod lesion and a portion of the underlying brown tissue of the ovary wall was similarly tested. The plates were evenly seeded with colonies similar to those from the other lesion.

On the same date two leaf lesions were cut out with flamed scissors, immersed first in alcohol to wet the surfaces and then in a 1:1,000 solution of mercuric chloride for a few minutes, rinsed in sterile water, and macerated in drops of sterile water on flamed slides. The loop-dilution plates from both lesions were evenly seeded with grayish-white colonies apparently identical with those in the plates from the pod lesions. Furthermore, 18 leaf lesions were similarly cut out, sterilized, rinsed, and planted in poured plates of potato-dextrose agar. Grayish-white bacterial growth occurred around nine of these. Poured plate isolations made on August 30 from leaf, pod, and stem lesions yielded a similar organism. Numerous transfers were made from typical plate colonies to dextrose-potato agar slants and all appeared to be similar. Successful atomizer inoculations were made in the field and greenhouse, and the organism was reisolated from the lesions produced.

Successful isolations were made later from seedling cowpeas grown in sterile soil from diseased commercial seed. The organism was obtained from lesions on cotyledons, first leaves, epicotyl, and hypocotyl, and from vascular infection in the epicotyl. In 1923 the

organism was isolated also from lima beans grown in home gardens and from those exposed to infection in the experimental plots at the Purdue Experiment Station.

For the subsequent detailed study three strains were used, one an isolation from a cowpea-leaf lesion made August 26, 1921, and two were reisolations made in September, 1921, from cowpea-leaf lesions produced by field inoculations. These strains were tested for purity by poured plates and for pathogenicity by inoculations.

MORPHOLOGY

The organism is a rather small rod with rounded ends, and occurs singly or in pairs. The rods stain more readily in gentian violet than in Ziehl's carbol fuchsin or rose bengal. In agar cultures 24 hours old the cells varied in width from 0.44 to 0.66 μ , and in length from 1.10 to 2.34 μ , with an average of about 0.54 by 1.47 μ .

To obtain actively motile cells for the flagella stain, a piece was cut out of a 48-hour-old culture on potato-dextrose agar at the base of the slant and dropped into a sterile water blank. After four hours an examination of a hanging drop showed an abundance of motile cells and indicated that the organisms had diffused throughout the liquid. Smears were made from this water culture, and the flagella were stained by Van Ermengem's method. One to five flagella were found at one or both poles. Considerable variation was found, but more frequently there were three or four flagella at one pole and fewer at the other. Five flagella were noted at each pole in some instances. In another culture the predominating condition was that of one or two flagella at only one end of the rod. A few flagella were measured and averaged 6.5 μ in length.

Endospores, capsules, and involution forms have not been noted. In water suspensions 30 hours old the cells become swollen and vacuolated. The organism is gram negative.

CULTURAL CHARACTERS

The organism grows well on potato agar with 1 or 2 per cent dextrose and no peptone, and this medium has been generally used. Equally good growth occurs on lima-bean agar without dextrose. Unless otherwise specified, the cultures were incubated at room temperature. The reaction of the media as expressed in Fuller's scale was adjusted by titration, using as the neutral point the first permanent but faint pink color with phenolphthalein. Inoculations were made from water suspensions unless otherwise stated. Cultures of *Bacillus coli* and *Bacterium glycineum* Coerper were carried in parallel series in part of the tests.

AGAR POURED PLATES.—On + 10 beef-peptone agar, colonies appeared in 24 hours; and in 56 hours surface colonies were 2 mm. in diameter, and round, raised, glistening, and grayish white. Submerged colonies were smaller and lens shaped. In six days surface colonies were round with an entire margin, raised, and smooth, with a finely granular internal structure showing faint concentric markings (pl. 4, A). The color was grayish white in reflected light and slightly fluorescent in transmitted light.

Surface colonies on potato-dextrose agar incubated four days at 27° C., were 3 to 4 mm. in diameter, and in one week were 5 to 7 mm. in diameter. The colonies were round, and the margin entire (pl. 4, A); the surface was smooth or concentrically ridged; and the elevation varied from raised to pulvinate, with the central portion often higher, making the colony umbonate. The colonies had a finely granular appearance, with a more or less concentric pattern, as

illustrated in Plate 4, C, and were grayish white in reflected light and greenish fluorescent in transmitted light. The colonies which emerge after the agar surface has dried slightly seem to pile up more conspicuously. Colonies on agar that has dried out to some extent have a scalloped margin and sculptured surface, as shown in Plate 4, B. Submerged colonies are lens-shaped, white, and very small (pl. 4, A). Colonies on the underside of the agar are thin, transparent, and greenish fluorescent. The agar is unchanged in color. Only a slight odor is noticeable.

AGAR STABS.—In +10 beef-peptone agar, growth along the stab was scanty and filiform, and on the surface was restricted but piled up. In potato-dextrose agar, growth occurred in the stab along the upper part only, and this was slight; but on the surface the growth was abundant, spreading, flat, and dull with a rugose zone. Growth on this medium was much more vigorous than on the beef-peptone agar.

AGAR SLANTS.—On +10 beef-peptone agar, the growth was moderate, spreading, flat, smooth, and grayish white. On potato-dextrose agar the growth was abundant, spreading, flat, dull, finely rugose, and grayish white. The margin was entire, with a definite beveled border. There was no change in the color of this medium in either case. On lima-bean agar a slight greenish pigmentation of the medium occurred.

GELATIN PLATES.—In two days flat, circular, white colonies, producing saucer-shaped zones of liquefaction, were present. Liquefaction had proceeded to completion the third day.

GELATIN STABS.—The liquefaction was napiform in 2 days, infundibuliform in 3 days, stratiform in 7 days, and complete in 14 days, with a white flocculent precipitate.

POTATO CYLINDERS.—On steamed potato cylinders growth was rapid and abundant, grayish white, and somewhat iridescent, smooth, and glistening, and had spread over all the moist surface of the substratum. There was no change in the color of the potato tissue.

MILK.—Clearing of the milk without coagulation began at the top in 2 days and was complete in 33 days. Throughout this period the cleared liquid was of a pale greenish-yellow color, and the liquid became viscid or gelatinous in consistency.

LITMUS MILK.—Pale-blue litmus milk was completely decolorized in seven days, but no pink color appeared. Digestion proceeded as noted above and was complete in 26 days. The cleared liquid was slightly yellowish green.

BROM CRESOL PURPLE IN MILK.—Brom cresol purple produces a light bluish color in milk and becomes yellow if the hydrogen-ion concentration is increased. Seven days after inoculation the color was unchanged, except that it was even more purplish in the upper cleared portion. After 21 days the blue color remained. Accordingly, there is no acid production in milk.

METHYLENE BLUE IN MILK.—In this medium a deeper blue color was noted at the end of two days, while after seven days the milk was completely decolorized except for the cleared portion at the surface, which was greenish. After 33 days the color was maize yellow, except for a thin greenish layer at the surface.

REDUCTION OF NITRATES.—In fermentation tubes containing 1 per cent potassium nitrate in a 2 per cent Difco peptone solution, there was good growth in the open arm and none in the closed arm. No gas was formed. At the end of a month the liquid in the open arm showed a slightly yellowish green. Test-tube cultures in the same medium were tested with Trommsdorf's reagent at 14 and 33 days after inoculation, and no nitrites were detected. With Nessler's reagent, a strong positive test for ammonia was obtained. Apparently nitrates were not reduced, and ammonia probably was produced from the peptone.

CARBON METABOLISM.—To test for acid and gas production with different carbon sources, 2 per cent solutions of dextrose, saccharose, maltose, lactose, mannite, and glycerin were made up in a 2 per cent Difco peptone solution. Cultures were run in duplicate, first in the ordinary U type of fermentation tube and later in a simpler and more convenient type of fermentation tube (9, fig. 47) consisting of a smaller inverted test tube within a larger one, a type very satisfactory where it is not necessary to measure the quantity of gas. In all cases there was abundant growth in the open arm and none in the closed arm. No gas was formed. Titration with N/20 sodium hydroxide at the end of 17 days in one series and 33 days in the other revealed no marked change in acidity as compared with the sterile control tubes. The lactose and glycerin cultures were slightly less acid than the controls in one series.

In order to determine more accurately the changes in true acidity in these media, three series of six test tubes each were made up, one series containing brom cresol purple, one brom thymol blue, and the other phenol red in a concentration of 0.0016 per cent. These media were adjusted to about P_H 7.3 as indicated by the blue-green color of brom thymol blue and the red of the phenol red. The dextrose and saccharose cultures with brom thymol blue and phenol red became yellow (more acid) seven days after inoculation. All the other cultures slowly became less acid than the controls. There was, therefore, acid production from dextrose and saccharose, but none from the other carbon sources. Probably the decrease in true acidity was due to ammonia produced from the peptone.

AGAR WITH SUGARS.—In litmus-dextrose, litmus-maltose, and litmus-lactose agar slant cultures there was no evidence of acid production. In two days the maltose and lactose cultures were blue under the stroke, and in seven days all cultures were bluer throughout than the controls. However, in a later repetition of this series, in which saccharose, mannite, and glycerin were also tested, a pink color developed in the dextrose and saccharose tubes, indicating acid production.

To obtain more accurate information on this point, the three sulphone phthalein indicators, brom cresol purple, brom thymol blue, and phenol red, were used in a triplicate series of slant cultures with dextrose, maltose, and lactose. These media were adjusted to a P_H of about 7.0 as evidenced by the grass-green color of brom thymol blue. In the dextrose cultures containing brom cresol purple and brom thymol blue a yellow color developed, indicating acid production. No increase of hydrogen-ion concentration was indicated at any time with the other two sugars and most of the cultures became slightly more alkaline. In a later repetition of this test, saccharose, mannite, and glycerin were also included and distinct acid production occurred only in the dextrose and saccharose cultures. Parallel series of cultures of *Bacillus coli* produced the yellow-acid color with all of the indicators. These tests show that there is acid production from dextrose and saccharose.

ACTION ON STARCH.—There were no signs of diastatic action on either potato or corn starch. No halos or cleared zones appeared about the colonies in plates of beef agar to which starch was added.

TESTS FOR INDOL, SKATOL, AND AMMONIA.—Cultures in beef-peptone bouillon gave no test for indol at 7, 26, and 33 day intervals when tested with potassium nitrite and sulphuric acid and no test for skatol when tested with nitric acid and potassium nitrite. A positive test for ammonia was obtained after 7 and 33 days with Nessler's reagent.

FERMI'S SOLUTION.—Growth occurred in Fermi's solution, accompanied by the formation of a greenish-yellow pigment throughout the medium.

USCHINSKY'S SOLUTION.—Good growth occurred in Uschinsky's solution in the case of two of the three strains tested, and a greenish-yellow pigment was produced.

COHN'S SOLUTION.—No growth occurred in Cohn's solution.

BLOOD SERUM.—The growth in stroke cultures on plain solidified blood serum in 10 days was abundant, spreading, flat, smooth, and glistening, and showed a brownish tinge. A slight liquefaction of the medium along the stroke was noted after 17 days, and at the end of 40 days there was a general liquefaction of the upper part of the slant, and a brownish-yellow discoloration of the medium.

On slants of Loeffler's blood serum, growth was more rapid than on plain blood serum, and in two days was abundant, spreading, flat, and rugose. In 10 days the medium showed a slight brownish color and slight liquefaction under the stroke. At the end of 40 days the medium was almost completely liquefied, and was clay color according to Ridgway's chart (16).

TOLERATION OF SODIUM CHLORIDE.—In tubes of beef-peptone bouillon neutral to brom thymol blue, 4 per cent of sodium chloride was tolerated and 5 per cent inhibited growth. In a series neutral to phenolphthalein, 5 per cent of sodium chloride was tolerated. Evidence was obtained that the use of hydrochloric acid in adjusting the reaction increased the inhibitory properties of sodium chloride.

TOLERATION OF ACIDS AND ALKALIES.—Tubes of beef-peptone bouillon were adjusted to +30, +25, +20, +18, +15, +14, +12, +10, +5, 0, -5, -10, -15, -20, -30, and -40 Fuller's scale by the use of hydrochloric acid and sodium hydroxide. The three strains grew in the +10, +5, 0, -10, and -15 tubes, and two strains grew in the +12 tubes. In a later series growth occurred in the +15 and -25 tubes. Growth seemed most vigorous in the +5 media. A greenish-yellow pigment was formed in the alkaline cultures.

In order to determine the tolerance of true acidity as indicated by the hydrogen-ion concentration, a duplicate series of beef-broth tubes was made up, one with hydrochloric acid and the other with malic acid, and adjusted by means of the sulphone phthalein indicators. No growth occurred in the tubes acidified to P_H 4.1 with either acid, but retarded growth occurred in both cases in the tubes acidified to P_H 4.5. Good growth occurred in the P_H 5.0 tubes. Growth occurred in tubes rendered alkaline to P_H 8.5 with either sodium hydroxide or ammonium hydroxide.

TEMPERATURE RELATIONS

The organism grows throughout a wide range of temperatures. Slant and plate cultures on potato agar incubated in moist chambers at 3° C., 9°, 12°, 15°, 20°, 23°, 27°, 30°, and 35° showed that the organism did not grow at 3° or 35°, but grew slowly at 9°, and 12°, moderately at 15°, 20°, and 35°, and rapidly at 23°, 27°, and 30°, with a fairly distinct optimum at 27°.

In determining the thermal death point, water suspensions from agar slant cultures were subjected to 10-minute exposures to a series of temperatures in a water bath and tested by loop transfers to agar slants (12). The thermal death-point was found to lie between 49° and 50° C.

EFFECT OF FREEZING

Heavy water suspensions from slant cultures were placed in test tubes, frozen in an ice-salt mixture, and held in a refrigerator. At intervals tubes were removed and thawed out and plates poured. The approximate number of living bacteria per cubic centimeter as indicated by the plate counts is shown in Table I.

TABLE I.—Effect on bacteria of freezing in water

Time frozen	Approximate number per cubic centimeter		Time frozen	Approximate number per cubic centimeter	
	Strain A	Strain B		Strain A	Strain B
0 ^a	12, 635, 000	106, 714, 000	5 days.....	2, 000	149, 000
4½ hours.....	3, 192, 000	35, 285, 000	8 days.....	250	2, 400
1 day.....	2, 777, 000	13, 050, 000	11 days.....	360	540
2 days.....	27, 000	1, 224, 000	15 days.....	20	0
4 days.....	4, 000	21, 000			

^a Original suspension.

The results in Table I show that the organism is slowly killed by freezing in water.

EFFECT OF SUNLIGHT

Plates poured from tubes inoculated with a suspension of the organisms, and each partly shaded with black paper attached to the glass, were placed on a cake of ice and exposed to the afternoon sun for periods of varying length. Ten and fifteen minute exposures did not reduce the number of colonies, while 25, 30, and 45 minute exposures greatly reduced the number of colonies, and exposures of 60 minutes or more resulted in complete sterilization.

RESISTANCE TO DESICCATION

The organism is very sensitive to desiccation on glass. Drops of a water suspension of the organisms were allowed to dry on sterile cover slips, and tests were made by inserting the cover slips into agar

slants. No growth was obtained from smears that had been dry 40 minutes, and in most instances no growth was obtained from smears that had just dried.

To test the resistance of the organism to drying on cowpea seeds, small quantities of seed were placed in Petri plates, moistened, sterilized in the autoclave, and allowed to become air-dry. A water suspension of the organisms was then poured over this sterilized seed and allowed to dry. The seed was tested at intervals by planting in agar-poured plates and the organisms were found alive during the following four months. Furthermore, it has been found that the organism lives over winter in the seed, so that it is evident that it is highly resistant to drying on and in cowpea seeds.

Tisdale and Williamson (24, p. 150) found the organism alive in lima-bean leaves dried 2½ years. In the writers' experience the organism has not generally been found viable in the older tan-centered leaf lesions on cowpeas; it has a tendency to be rather short lived on potato-agar slants, much more short lived than certain yellow organisms such as *Bacterium phaseoli* E. F. S. and *Bact. vesicatorium* Doidge.

TAXONOMY

The organism causing bacterial spot of cowpea is not identical with any of the previously described bacterial parasites of cowpea, being clearly differentiated in its chromogenesis and other salient characters. It was briefly described in March, 1923, (6), and given the name *Bacterium vignae* n. sp. Upon the appearance of this preliminary note the writers received a letter from W. B. Tisdale calling attention to the marked similarity of this organism to the causal organism of the lima-bean disease, a description of which was in press at that time and appeared four months later (24). The causal organism of the latter disease was designated *Bacterium viridifaciens* n. sp.

The lima-bean disease was found rather commonly in gardens about La Fayette, Ind. The causal organism was isolated from pod lesions and proved to resemble closely the cowpea organism. With the organism isolated from lima beans, abundant and typical infection of cowpea seedlings grown under a cloth cage in the field was obtained August 15, 1923, along with infection of lima-bean seedlings. In another cage, typical infection of lima-bean seedlings was obtained with one of the strains isolated from cowpeas. Later a culture of Tisdale's organism was obtained from the University of Wisconsin, and with it successful inoculations of cowpea seedlings with the production of characteristic bacterial spot lesions were obtained in the greenhouse in April, 1924. These cross inoculations indicated beyond doubt the identity of the two organisms.

In the meantime, however, the cowpea strain, the strain isolated from lima beans at La Fayette, Ind., and the original lima-bean strain from the Wisconsin laboratory were carefully compared as to a large number of cultural and physiological characters and found practically identical. These tests included the ordinary media such as gelatin, agars, and milk with indicators, agar and bouillon with the six carbon sources and the three hydrogen-ion indicators previously mentioned, toleration of sodium chloride, toleration of acidity, blood media, and Cohn's, Fermi's, and Uschinsky's media. Furthermore, the published description of *Bacterium viridifaciens*

is practically in complete accord with the writers' earlier description and the data herein recorded. While the writers failed to detect an increase in acidity in the dextrose and saccharose fermentation-tube cultures by titration, they obtained striking evidence of acid production with these sugars in the cultures containing the hydrogen-ion indicators. The only difference in group number is due to the writers' failure to class the organism as fluorescent. Since the cowpea and lima-bean organisms are thus shown to be identical, the binomial *Bacterium viridifaciens* becomes synonymous by priority rules with the previously published name, *Bacterium vignae*.⁴ (Group Number, 5322-31131-2232).

This organism shows some resemblance to certain other plant pathogenes, such as *Pseudomonas pisi* Sackett (17), which it resembles culturally but from which it differs in morphology and pathogenicity, and *Pseudomonas maculicolum* McCulloch (13), which it resembles culturally and in type of lesion produced but from which it differs in pathogenicity. It differs both in culture and in pathogenicity from *Bacterium glycineum* Coerper, *Bact. trifoliorum* Jones, Williamson, Wolf, and McCulloch, and *Bact. tabacum* Wolf and Foster, although the Virginia strain of *Bact. trifoliorum* was found to be pathogenic to lima bean and velvet bean (11, p. 486). The organism differs radically in morphology from *Aplanobacter stizobii* Wolf (26), the causal organism of bacterial leaf spot of the velvet bean. Attempts to infect cowpeas with *Pseudomonas pisi* have given only negative results, corroborating Sackett's (17, p. 18) conclusion that cowpeas were not a host for that organism.

PATHOGENICITY OF CAUSAL ORGANISM

Infection of young, healthy cowpea plants has been produced at will by spraying (from an atomizer) with a water suspension of a young agar slant culture. This has been done in the field and more frequently in the greenhouse, where the plants could be held in a moist chamber for one or two days after the inoculation. The same has been done with young lima-bean plants.

The writers have found, as did Tisdale and Williamson (24), that the organism tends to lose its virulence in culture rather rapidly and that best results are obtained with recently isolated or reisolated strains.

The incubation period for leaf infection of cowpeas and lima beans under greenhouse conditions is two to four days, and in the field in August lesions have become visible two days after inoculation. Wounds are not necessary for infection, and the abundance of lesions indicates that the mode of entry into the host tissue is undoubtedly stomatal.

Cowpea seedlings and the younger leaves of older plants have proved much more susceptible to infection than older parts of the plant. It seems possible that this fact may be correlated with a lower hydrogen-ion concentration in the young leaves, a condition found to exist in clover by Haas (8, p. 350) and later in pole beans by Gustafson (?). In the writers' work with bacterial spot of

⁴ According to Migula's classification, Buchanan's revision (2, p. 48), and the revision adopted by the committee of the Society of American Bacteriologists (21, p. 203), the combination would be *Pseudomonas vignae* n. sp., while in a later report of another committee of the same society (22, p. 188), the name has already been changed to *Phytomonas vignae*.

tomato (5, p. 148) a correlation was found between the resistance of ripe fruit to infection and the higher hydrogen-ion concentration in the ripe fruit as compared with the leaves and green fruit, and in that disease also the younger parts of the plant were more susceptible to infection.

Clevenger (4, p. 238) found that the hydrogen-ion concentration of cowpea leaves was slightly lower than in the stems, and that it showed a diurnal variation (4, p. 233). A maximum concentration (P_H 5.27) occurred in the leaves at 10.30 a. m. and a minimum (P_H 5.81) at 1 a. m., while in the stems a maximum (P_H 5.04) occurred at 6.30 a. m. and a minimum (P_H 5.32) at 9 p. m. The writers in their cultural tests found that the parasite tolerated a P_H of 4.5 and grew well at P_H 5.00, so it is evident that the cowpea leaves and stems are well within the limit of tolerance. The lower acidity of the leaves in the night may favor infection at that time.

Atomizer inoculation has been successful upon all of the varieties of cowpea, sieva bean, and lima bean tested, including most of the varieties mentioned in the previous discussion of hosts of this disease, and upon the catjang, hyacinth bean, adsuki bean, and Florida velvet bean (varieties, Bunch and 100-Day). The organism was successfully reisolated from each host species. The three *Phaseolus limensis* varieties—Burpee, Fordhook, and Large White Pole—have appeared to be more susceptible than Henderson's Bush Lima, which, according to Bailey (1, p. 396), is a different species. In fact, in the writers' field plots in 1924, Henderson's Bush Lima showed considerable resistance. While the cowpea varieties have been considered equally susceptible, the varieties Early Red, Clay, California Blackeye, Iron, Groit, and Whippoorwill were more severely diseased than any of the other 12 varieties in the 1923 plots. In the 1924 plots the varieties Early Red, Groit, Whippoorwill, New Era, and Catjang were more severely diseased than Blackeye, Iron, and Early Buff. Rather light natural infection occurred on the foliage of velvet beans grown adjacent to cowpeas in 1924, and the organism isolated resembled *Bacterium vignae* in culture, and in inoculation tests produced typical lesions on cowpeas.

The organism isolated from the lesions on *Desmodium canescens* resembled the cowpea organism in culture, and in cross-inoculation tests in a field cage and in the greenhouse produced typical bacterial spot infection on cowpeas. The organism was successfully reisolated, and although its morphology and cultural characters have not yet been studied by the writers, it seems safe to assume that the *Desmodium* organism is *Bacterium vignae*. The importance of this weed as a possible source of infection is not known.

Unsuccessful attempts have been made to inoculate garden beans (five varieties), soy beans (five varieties), broad bean, sweet pea, peas (seven varieties), lupine, clovers (white, Bokhara, crimson, annual sweet, mammoth red, and alsike), cauliflower, tobacco, tomato, and potato.

RELATION OF PARASITE TO HOST TISSUE

As atomizer inoculation of cowpeas without wounds is successful, it seems likely that the tissues are entered by way of the stomata. Tisdale and Williamson (24, p. 151) found that the lima-bean leaf was invaded through the stomata. Microscopic examination of the

leaf lesions shows that the invasion is intercellular and tends to be restricted to the mesophyll during the early stages. In fact the mesophyll may be rather extensively involved without any apparent injury to the palisade tissue. The advance invasion is in the mesophyll layers adjacent to the lower epidermis. The cells involved become a dense reddish brown and soon collapse. The lower mesophyll layers may thus become discolored and collapsed while the upper mesophyll layers and the palisade layer remain apparently uninjured. Later the entire thickness of the lamina is killed and dries out at the center of the lesion.

In cowpea seedlings vascular infection has been frequently observed. The organism may gain entrance to the vascular system from infected cotyledons, epicotyl lesions, petiole lesions, or from lesions on the veins of the leaf. Reddish-brown bundles were traced from the infected cotyledon or epicotyl down into the hypocotyl and up through the epicotyl into the leaf veins (pl. 4, F). In water mounts, the bacteria were seen to ooze from the cut ends of these reddish-brown spiral vessels of the epicotyl, and in cross section these vessels appeared to contain bacteria. The organism was cultured from these internally discolored vascular bundles at some distance from the lesion where it had entered the host. From lesions on the larger veins of the seedling leaves, vascular infection may extend outward a distance of a centimeter or more, and in some cases the reddish-brown vascular elements, being in the xylem, are more clearly visible from the upper leaf surface.

A microscopic examination of unstained razor sections of these internally discolored veins showed that the reddish-brown color was localized in the walls of certain spiral vessels and that this color was very intense, constituting an excellent stain for the walls of the vessels even under the higher powers of the microscope. The stain was also noted in the tubes of the small veinlets in the affected areas. Stained strips of collapsed cells also occurred in the dorsal cortex of the veins and in the mesophyll along the veins.

When the freshly cut edges of leaf lesions were examined in water mounts, the bacteria were seen to ooze from the cut ends of the veins. In cross section the bacteria were noted in the intercellular spaces of the parenchyma of the veins but were not actually demonstrated within the spiral tubes at any distance from the leaf lesion. The organism very evidently shows a preference for the vein tissues and travels more rapidly in the veins than in the mesophyll. In older leaves the tendency for internal vascular invasion is not as marked, although linear surface lesions of considerable length often occur along the veins (pl. 1, A, B; pl. 2, D).

Shallow surface lesions on the epicotyl, hypocotyl, and petioles were found to be limited to the outer cortical layers. The cells in the epidermal and cortical layers were collapsed and dense dark red in color. In large hypocotyl lesions there was evidence of deeper invasion, not in a solid front, but in the shape of ramifying intercellular penetration. There was marked hypertrophy of the cortical cells immediately beneath the epicotyl lesions, and in many cases rather extensive hyperplasia had resulted from the formation of cross walls in these enlarged cells, suggestive of an attempt to occlude the lesion with a cork layer. Very early infection of the

epicotyl results in linear lesions occupying deep grooves and in cross section it was seen that these lesions extended inward through the cambium to the pith and were accompanied by extensive hyperplasia of the latter tissue. These lesions result in a break or uncompleted segment in the vascular cylinder, and, as a result of enlargement of the rest of the epicotyl tissues, become sharply sunken channels or grooves. Shallower lesions were noted which had not interrupted or impaired the cambium and which had occasioned a marked hyperplastic response.

TRANSMISSION OF THE DISEASE THROUGH SEED

Owing to the abundance of cowpea-pod lesions, many of which were found to penetrate through the pod tissues into the seed, actual infection of the seed by this organism may occur very readily and very generally (pl. 3, C and E). Furthermore, it was found that the organism could endure long periods of desiccation on the surface of the seed, being found viable after four months of drying.

In order to determine the possibility of seed transmission of the disease, a number of seeds selected from diseased pods collected in the fall of 1921 were sown in pots of sterilized soil in the greenhouse on March 8, 1922. Among 123 seedlings grown from seeds borne directly under pod lesions, 18 showed bacterial spot infection; and among 198 seedlings grown from seeds not borne directly under lesions, 5 showed infection. These tests proved that the disease was carried with the seed and that infected or contaminated seed gave rise to diseased seedlings.

This primary infection consisted of lesions on the hypocotyl, epicotyl, cotyledons, and first leaves. In some cases lesions had originated at the point of attachment of a cotyledon and extended down into the hypocotyl (pl. 3, F) and up into the epicotyl. Infected cotyledons tended to remain attached longer than normal ones. In one case there was one lesion on each first leaf, each located on the under side of the midrib at a point corresponding exactly with the location of the other. Considerable local vascular infection and localized wilting occurred among these seedlings. The organism was successfully isolated from these seedlings.

Similar tests in pots of sterilized soil were made with 20 lots of commercial seed from two sources representing 16 varieties. The shriveled, discolored seeds were tested separately. The seed was planted April 26, 1922, and the results as noted on May 12 are given in Table II.

The results given in Table II show that the infection was present in 15 of the 20 commercial seed lots tested, and occurred in the seed from both sources and in seed of normal appearance as well as in shriveled or discolored seed. Most of the infected plants represented primary seed-borne infection. The organism was isolated from a number of the seedlings. The high incidence of infection in some of the seed lots, coupled with the large proportion of the lots showing infection, indicates the great extent to which the disease may be present in cowpea seed. The appearance of a seed can not be depended upon to indicate whether or not it may transmit the disease:

TABLE II.—*Tests of commercial cowpea seed samples for the presence of bacterial spot infection*

Variety	Source ^a	Seeds normal in appearance		Seeds shriveled or discolored	
		Number of plants	Number infected	Number of plants	Number infected
Early Red.....	A	14	0	32	0
Early Black.....	A	19	0	22	2
Early Buff.....	A	28	0	32	2
Conch.....	A	12	0	20	0
Taylor.....	A	23	0	35	1
Brabham.....	A	28	1	37	0
California Blackeye.....	A	23	16	24	11
Vigna catjang.....	A	18	0	34	2
New Era.....	A	28	1	36	4
Do.....	B	13	0	19	0
Whippoorwill.....	A	30	0	24	4
Do.....	B	21	0	16	0
Iron.....	A	19	4	31	10
Do.....	B	20	0	23	0
Groit.....	A	29	7	35	0
Do.....	B	17	0	14	0
Red Ripper.....	B	21	0	29	2
Black.....	B	24	0	21	1
Wonderful.....	B	28	0	30	6
Clay.....	B	26	6	23	3

^a Two sources designated A and B.

To determine the effect of age of the seed upon the presence of bacterial spot infection, 125 plants were grown in sterilized soil in the greenhouse in March, 1923, from the same lot of seed of the Whippoorwill variety with which the experimental plot had been planted in 1921. The disease had appeared in this field plot very evidently as a result of seed-borne infection, but did not appear on the greenhouse plants grown in 1923. This would indicate that the extra two years of storage had eliminated the organisms.

TABLE III.—*Effect of one extra year of storage on bacterial spot disease transmission in infected seed in certain commercial seed lots listed in Table II, determined by planting some of the seed in sterilized soil in the greenhouse in March, 1923*

Variety	Source of seed ^a	Planted April, 1922		Planted March, 1923	
		Number of plants	Number infected	Number of plants	Number infected
Early Black.....	A	41	2	66	0
Early Buff.....	A	60	2	37	0
Vigna catjang.....	A	52	2	131	0
New Era.....	A	64	5	63	0
Iron.....	A	50	14	35	0
Groit.....	A	64	7	89	0
Red Ripper.....	B	50	2	19	0
Wonderful.....	B	58	6	29	0
Clay.....	B	49	9	23	0

^a Two sources designated A and B.

The results in Table III indicate that one year of storage had eliminated the seed infection in every variety tested. While the age of this seed is not known with certainty, it is very probable that it came from the 1921 crop. Although these pot tests represent only a small number of seed, it would seem that seed two and three years old is freer from infection than one-year-old seed, as was found by Rapp (15) in the case of bean bacterial blight.

GENERAL INVESTIGATIONS.

CONTROL

Since the disease is seed borne in the case of cowpeas, prevention of it would primarily require the use of disease-free seed. Disease-free seed can be obtained in small quantities by seed selection from disease-free pods. The use of two or three year old seed should result in decreased primary infection.

In 1924 the disease was found on volunteer plants in a plot that was in cowpeas the year before. Therefore crop rotation would seem advisable.

SUMMARY

Bacterial spot of cowpea is characterized by reddish-brown lesions on the leaves, stems, pods, and seeds.

The hosts include all varieties of cowpea tested, catjang, hyacinth bean, asparagus bean, adsuki bean, velvet bean, lima bean, bush lima bean, dwarf sieva bean, and the common weed, tick trefoil. Henderson's Bush Lima bean has shown some resistance.

On cowpeas the young growing organs are most susceptible, and infection of such organs results in considerable distortion and shattering of the leaves, deformity of the pods, and discoloration and stunting of the seeds. In seedlings and young leaves localized vascular infection and localized wilting may occur.

The causal organism is a motile rod bearing one to five flagella at one or both poles, first described as *Bacterium vignae* n. sp. It is identical with *Bacterium viridifaciens* n. sp. described by Tisdale and Williamson.

The colonies on agar are grayish white and, in transmitted light, slightly fluorescent. Gelatin is liquefied.

Acid is produced only with dextrose and saccharose. Greenish pigment formation occurs in milk, alkaline broth, and in Fermi's and Uschinsky's solutions.

Atomizer inoculation without wounds is successful. The invasion in cowpea leaves is intercellular, and the mesophyll is most extensively involved. Vein tissues are preferred and vascular invasion may occur in seedlings and young leaves. Epicotyl lesions may be accompanied by hypertrophy and hyperplasia of the underlying cortical or pith cells.

Cowpea-pod infection results in seed infection. Seeds from infected pods gave rise to infected seedlings when planted in sterilized soil. Commercial seed of a number of cowpea varieties planted in sterilized soil also gave rise to infected seedlings. Similar seed stored an extra year seemed to be comparatively free from infection.

As control measures with cowpeas, the selection of seed from disease-free pods, or the use of seed two or three years old, and crop rotation are suggested.

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THE INFLUENCE OF CERTAIN ENVIRONMENTAL AND CULTURAL CONDITIONS ON FRUIT-BUD FORMATION OF PEAR AND APRICOT¹

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INTRODUCTION

The pear and apricot fruits are formed from flower buds differentiated during the summer previous to blossoming. It is generally assumed that fruit-bud formation can be influenced only by operations performed before the time of differentiation. If this is true, the orchardist is not only interested in the approximate time of differentiation, but also in the influence which certain cultural operations, such as pruning and irrigation, may have on the time and subsequent development of fruit buds. To add to the present knowledge of flower-bud formation, and to obtain information which might be of immediate practical value to the fruit industry of California, an attempt has been made to answer the following question:

What influence do (1) different degrees of severity of dormant pruning, (2) irrigation, and (3) climatic conditions in the coastal valleys, interior valleys, and foothills have upon the time of differentiation and the rate of development of the fruit buds of the pear and apricot?

As far as the writer knows, the only exact data which had been collected with regard to fruit-bud formation in California previous to the present investigation were those that dealt with average interior-valley conditions, no attempt having been made to determine the influence that such cultural practices as pruning and irrigation might have upon the time of differentiation and subsequent development of fruit buds. Likewise, data collected in the Eastern and Northwestern States possibly do not apply directly to California conditions, and furthermore, these other investigations have devoted little attention to the special problems involved here, since irrigation with them is not a common practice and neither does there exist such a diversity of pruning methods as in California.

Pruning, commonly recognized as a necessary orchard operation, is one of the most expensive and time-consuming practices of the California orchardist. In this State there are practically as many pruning methods as fruit-growing sections. Until recently the common practice in California has been to severely head back at the dormant pruning. A radically different method is rapidly gaining favor. This system consists essentially of thinning-out only and is popularly known as "long" pruning as opposed to the method of heavy heading-back or "short" pruning.

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Because of the comparatively light rainfall in winter and complete absence of rain in summer, irrigation is, with the exception of a few limited areas, a necessity for successful orcharding in California.

The problem of fruit-bud formation in California is further complicated by the fact that here latitude means little or nothing in determining climate. Isotherms normally run east and west. In California, with the high Sierra Nevada mountains on the east, the Pacific Ocean on the west, and minor mountain ranges on the north, south, and west, these isotherms are deflected so that their general direction is north and south (fig. 1).

In California there are three distinct climatic belts differing in degree rather than kind. These are coast, valley, and mountain, depending on distance from the ocean, location with reference to

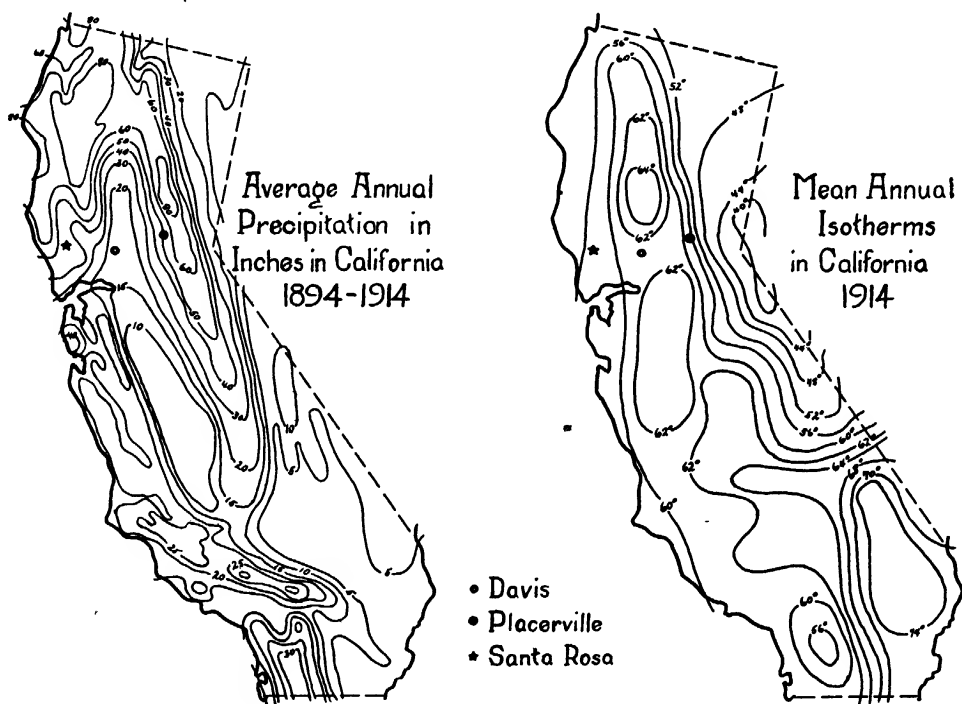


FIG. 1.—Outline map of California, showing average annual precipitation in inches for 20-year period 1894–1924; isotherms for mean annual temperatures in 1914; also the points from which fruit buds were collected

mountain chains, and altitude for their climatic differences. Fruit-growing sections are found in all of these belts.

The fruit-growing districts of the Pacific coast are located in protected places just back of low mountains, and are designated as coastal valleys. As compared with the interior valleys, these coastal valleys are characterized by warmer winters, cooler summers, more abundant winter rainfall, slightly more humidity in summer, and frequent fogs.

The interior valleys have the following general climatic characteristics: Higher summer and lower winter temperatures than the coastal valleys, abundant winter rainfall in the north decreasing rapidly southward; very dry air, free from fogs in the summer and with almost constant sunshine; occasional strong, hot, desiccating winds from the north in summer, and cold north winds in winter.

On the way up the foothills surrounding the valleys the seasons become increasingly distinct, mildly cold winters and warm summers being experienced. Hardy apples and pears grow well up to 4,000 feet elevation or somewhat higher. However, the chief fruit-growing sections of the mountains are found in the foothills at elevations of not higher than 2,500 feet. The climate in the foothills is a modification of the interior-valley climate. Temperatures are slightly lower, and the rainfall increases at the rate of about 1 inch to each 100 feet of elevation (fig. 1).

MATERIAL

The pear and apricot were chosen for study. These fruits were selected not only because of their commercial importance in California, but because one was a pome and the other a drupe. It was thought that other fruits of these classes might tend to be influenced in much the same way even though differentiating at very different times.

Pear and apricot buds were collected from three typical fruit-growing districts, namely: Sonoma County north and east of Santa Rosa, as representative of coastal valley climatic conditions; university farm at Davis, Yolo County, as representative of interior-valley climatic conditions; and Eldorado County east of Placerville, as representative of foothill climatic conditions.

Buds were collected in Sonoma County from Bartlett pear trees growing under three cultural conditions—heavily pruned and irrigated, heavily pruned and nonirrigated, and lightly pruned and nonirrigated. The trees were typical, heavily pruned specimens in good health, growing on Dublin loam, and were about 20 years old. The non-irrigated and lightly pruned tree was in good health, 8 or 10 years old, and was growing on yellow clay loam.

Bartlett pear trees growing on the university farm, Yolo County, under these three conditions were compared: Lightly pruned and irrigated, heavily pruned and irrigated, lightly pruned and non-irrigated. The first two mentioned were within 50 feet of each other and growing under apparently identical field conditions, except for pruning treatment. The soil was a Yolo sandy loam. The heavily pruned tree was typical, but the lightly pruned one was perhaps more vigorous than the average tree thus pruned. The lightly pruned and nonirrigated tree was located about one-quarter mile from the others, on Yolo clay loam, but under much the same conditions except for irrigation. The trees were all 7 years of age, and showed vigorous growth.

Collections of buds were made from only one tree in Eldorado County, at an elevation of approximately 2,400 feet. The tree was lightly pruned and irrigated, 7 or 8 years of age, and in a vigorous condition.

Apricots are not widely grown in Sonoma and Eldorado Counties, and it proved difficult to find suitable examples of this fruit under coastal valley and foothill conditions. However, collections were taken from the best trees that could be found in these locations.

The Royal apricot tree chosen in Sonoma County was 10 or 12 years old, lightly pruned and nonirrigated, and grew on a gravelly, fine, sandy loam.

Buds from Royal apricot trees growing under the three following conditions on the university farm were compared: Lightly pruned and nonirrigated, heavily pruned and irrigated, lightly pruned and irrigated. Each tree grew near and under the same cultural treatment as the pears described above. All were typical except the lightly pruned and irrigated tree, which apparently lacked vigor.

Few apricot trees could be found at the relatively high elevation in Eldorado County. The tree selected was thought to be a specimen of the Royal variety. It was an aged tree growing at an approximate elevation of 2,200 feet, and had received little or no pruning and but limited irrigation.

All trees in the experiment were under clean culture, with the exception of the apricot last mentioned.

Collections were made on or near July 10 and 20; August 1, 10, and 20; September 1 and 15; October 1 and 15; and November 1 from all trees except those in Eldorado County, where there were none taken July 10. In addition to these, samples were taken from all the trees at the university farm on December 1, and from the lightly pruned and irrigated pear tree and from the heavily pruned and irrigated apricot tree at intervals of from one to two weeks from January 1 until blossoming time.

METHODS

All buds were collected from fruit spurs not more than 3 inches long, and from all sides of the tree. About 30 buds were taken from each tree at each collection. Since fruit buds only were desired, the largest, plumpest pear buds and double and triple collateral buds of the apricot were taken. In the case of the apricot, contrary to the published inferences of Gourley (8),³ Bailey (1 p. 38) and others, the middle bud is not always a leaf bud. Many of these collateral buds were sectioned as a unit and all found to be flower buds.

Upon removal from the tree, the buds were immediately put in a formalin-alcohol killing and fixing solution, in which they were kept until ready to use. In later collections the outside scales were removed before killing, and with pears even the top part of the individual flower buds was cut away to eliminate the abundant hairs which hung on the knife edge of the microtome and prevented the proper ribboning of the paraffin mounts.

The major part of the sectioning was accomplished by the paraffin method as described by Chamberlain (2). The outstanding departures from Chamberlain's methods were less gradual dehydration and the holding of the material in the alcohol solutions for a shorter time. Safranin dissolved in alcohol and gentian-violet in clove oil proved to be the most satisfactory combination stain. Light green with safranin and Delafield's haematoxylin with safranin were tried but found less useful. Haidenhain's iron-alum haematoxylin proved to be useful as a nuclear stain but difficult to handle. Coplin jar tops, inverted and the inside surface coated with a thin film of glycerine, proved useful as embedding molds. Sections from 5 to 12.5 microns in thickness were cut. The thicker sections proved more satisfactory for photographing gross morphology. The thinner

³ Reference is made by number (*italic*) to "Literature cited," p. 882.

sections were made for the cytological study of pollen and ovary development. Only sections showing the floral parts were retained and mounted.

After the middle of September the hairs on the inner side of each bud scale became so numerous it was impossible to cut the pear buds embedded in paraffin. It therefore became necessary to employ "Gibson's rapid process" of the colloidin method as described by Lee (11). Drinkard (3) was able to cut all collections of the Kieffer pear in paraffin, but it is the writer's belief that the Bartlett has a greater abundance of hairs than the Kieffer. After the January 1 collection it was possible to continue the paraffin method, since the flower-buds had then become large enough to permit the removal of the hairs without serious injury to the floral parts.

RESULTS

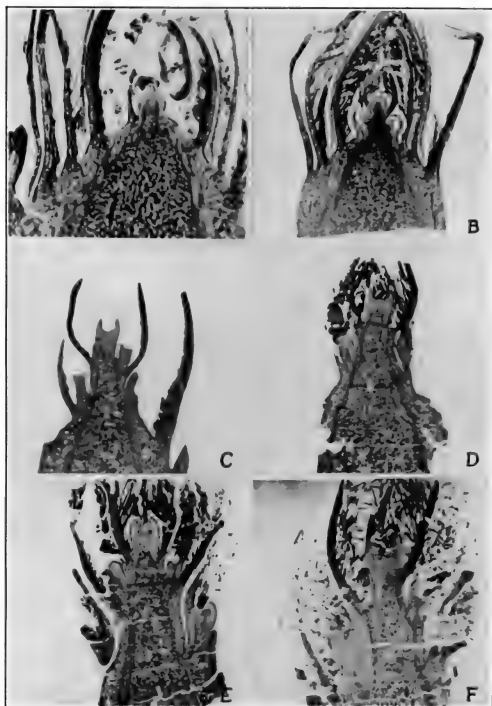
DIFFERENTIATION AND DEVELOPMENT OF FRUIT BUDS OF THE PEAR

Fruit buds were found among the buds collected July 7, 1922, from a lightly pruned and irrigated pear tree on the university farm (interior-valley conditions). However, since these flower buds were only in the first stage of differentiation, it can safely be said that the Bartlett pear began fruit-bud formation the first week in July during the 1922 season. This finding is in accordance with that reported by Henderson.⁴ At this time the growing point of the bud had ceased to give off bud scales and had become elongated and flattened on top. In the course of the next 10 days this crown had lengthened materially, and part of the specimens in longitudinal section showed the large terminal and two smaller lateral primordial flowers in the form of blunt protuberances (pl. 1). Each infant flower was subtended by a bract or modified leaf. It might be noted that the vascular system of the spur advances very closely with the elongation of the growing points and is lost sight of only near the tips.

In the collection made July 31 many buds were cut in which the terminal primordium had developed into a cuplike receptacle by a growth of tissue from its edges. The side primordia did not show as great development, but some had small projections at the edge. The August 10 samples showed little advance, except that in a few cases small round swellings on the inner surface of the receptacle cup showed the start of petals in the terminal flower. The entire bud slowly enlarged during the following 20 days, the primordial petals of the terminal flower became more prominent, and their beginnings became evident in the laterals.

During September (pl. 2) the buds continued to enlarge slowly and the first whorl of stamens had their origin from the torus lining the inner side of the receptacle cup, immediately below the petals, and resembling the petals at the early stages. In nearly all cases the terminal primordial bud which was the oldest was the largest in size and farthest advanced. By October 30 the petals had lengthened and at least another whorl of stamens had made its appearance. It was not determined at what stage this whorl could first be seen, although there appeared to be much the same arrangement

⁴ HENDERSON, W. THE DIFFERENTIATION AND EARLY DEVELOPMENT OF THE FLOWER BUDS OF THE BARTLETT PEAR. 1916. [Unpublished thesis, Univ. Calif.]



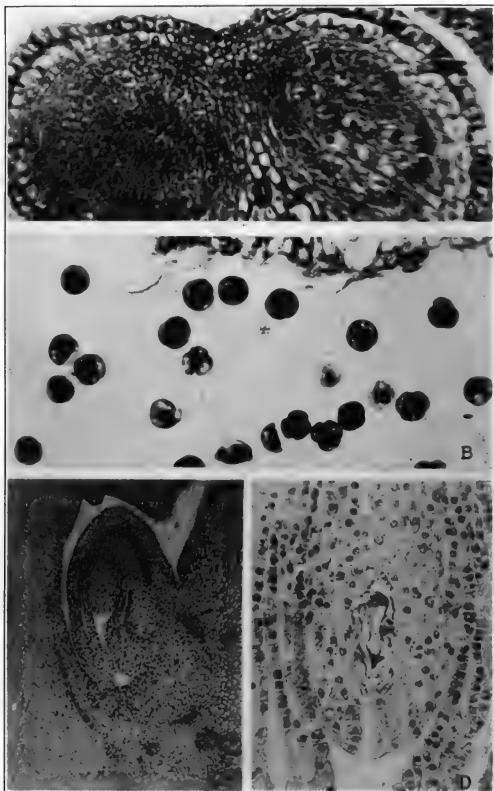
Photomicrographs of longitudinal sections through Bartlett-pear fruit buds collected from university farm orchard, showing the stage of development throughout the season of 1922-1923. (Approximately $\times 20$)

A, July 7; B, July 21; C, July 31; D, August 10; E, August 21; F, September 2



Photomicrographs of longitudinal sections through Bartlett-pear fruit buds collected from university farm orchard, showing the stage of development throughout the season of 1922-1923. (Approximately $\times 20$)

A, September 16; B, September 29; C, October 30; D, January 1; E, February 6; F, February 28



A.—Photomicrographs of cross-section through Bartlett-pear anther, February 21, 1923, showing the pollen mother cells. (\times approximately 450)

B.—Photomicrographs of cross-section through Bartlett-pear anther, March 12, 1923, showing mature pollen grains. (\times approximately 404)

C.—Photomicrographs of longitudinal section through the ovary of the pear, showing developing embryo sac. The nucellus is seen as an oval-shaped structure at the center surrounded by the inner and outer integuments. The large central cell of the nucellus is the embryo sac. (\times approximately 125)

D.—Same as C, enlarged. This evidently represents an early stage in the development, as only one nucleus is found. (\times approximately 355)

in later stages of the pear as Kraus (10) described for the apple. Small swellings on the bottom of the receptacle cup indicated the beginnings of carpels. Buds collected in December showed petals differentiated further and almost inclosing the stamens and carpels below. The stamens showed some differentiation into anthers and filaments, and styles were projecting upward from the carpels. In some cases cavities could be seen in the carpels.

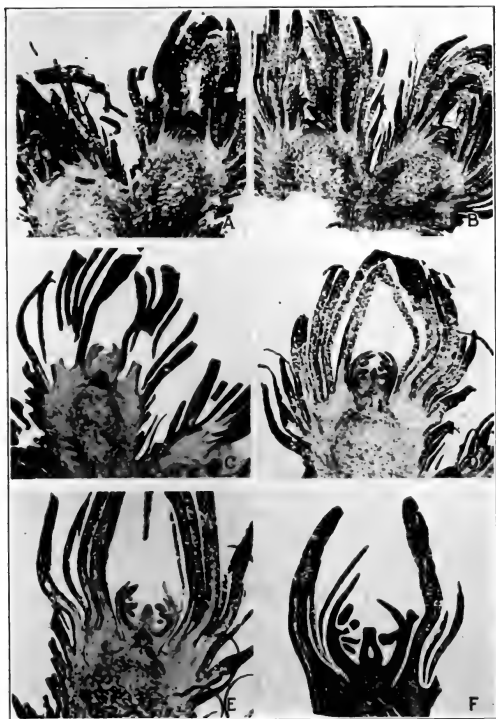
Mother cells (pl. 3) were clearly seen in the anthers of the buds gathered January 1. The mother cells were somewhat larger than the surrounding cells. They were located in the centers of the four lobes of the anthers and contained very large nuclei, staining dark red with safranin. The buds made little advance, except in size of anther, until about February 28. The mother cells had enlarged and become rounded, surrounding cells had differentiated into anther walls, and by the expansion of the anthers the mother cells had become more or less loosely associated. The five carpels had pushed up through the center of the bud into a well-defined pistil and two ovules could be seen as small knobs protruding from the enfolding placenta of the ovary. The petals completely covered over the other parts of the flower and appeared lanceolate in longitudinal section. Growth expansion was taking place in all parts and swelling of the buds could be observed by external examination in the field.

Well-defined tetrads, or clusters of the four granddaughter cells, were in evidence in material gathered March 8. This four-cell stage must be of short duration, because six days later each unit of the tetrad had broken away and was differentiated into a nearly, if not entirely, mature pollen grain.

On March 6 the stage of development of the anthers was much in advance of that of the carpels. The pollen grains were matured, whereas the ovules were at very early stages. One might be led to believe from this that the condition would result in a clear case of protandry. On the contrary, development in all parts of the pistil progressed from this date at an exceedingly rapid rate. From the small protuberances marking the start of the ovules on February 28, outer and inner integuments were well defined and inclosed the embryo sacs. Development in the remainder of the pistil was as rapid. The styles elongated greatly, and stigmas were differentiated, while enlargements of all parts took place.

DIFFERENTIATION AND DEVELOPMENT OF FRUIT BUDS OF THE APRICOT

The first collection of heavily pruned and irrigated Royal apricots on the university farm (interior-valley conditions) was made July 7. When these buds were sectioned it was found that the growing point was perfectly smooth, rounded slightly, and giving rise to bud scales from all sides. In this condition it was impossible to distinguish between future leaf buds and fruit buds. The same was true of all collections until July 31. At this date no definite signs of the initial fruit-bud stage were found, but the crown was giving rise to no more bud scales. On August 10 (pl. 4) the crown or growing point had become slightly elevated and flattened on top, while very small protuberances were observed at each side in longitudinal section, showing definite differentiation into fruiting buds. The sepal primordia were



Photomicrographs of the longitudinal sections through Royal-apricot fruit buds collected from university farm orchard, showing stages of development throughout the season of 1922-1923. (\times approximately 23)

A, August 10; B, August 21; C, September 16; D, September 29; E, October 13; F, October 30

the first to appear. Walker⁵ found differentiation started August 4 (1916) under similar conditions. It is interesting to note that the buds of a given date and condition that were observed were of surprisingly the same stage of development. As pointed out by Henderson,⁶ pear buds of a given collection varied widely in the stage of development.

Little advance could be seen in the material collected August 21, as compared with that of August 10, except that the small protuberances or sepal primordia were more pronounced. However, by September 2 considerable advancement had taken place. The sepals had pushed upward and inward at the top, forming a cup, while the whole bud had broadened, except at the base. Primordia of petals were present inside the cup. They arose from the inner surface of the cup or receptacle. Also, the pistil was prominent as a blunt swelling from the floor of the cup. By September 16 all parts had further enlarged, and in addition to the sepals and petals the outer whorl of stamens was present. By September 29 the second and third whorls of stamens were present and all were of apparently the same stage of development. At the same date, owing to this elongation, the petals were drawing together above the stamens.

All parts continued to enlarge during October. By October 30 the petals, narrowly attached at the base, appeared lanceolate in section. Stamens were well differentiated into filament and anther, while in the four lobes of the latter pollen mother cells were observed. The pistil had elongated and the style could be distinguished from the broadening ovary. The ovarian cavity was present, but as yet no ovules were evident. By December (pl. 5) the petals had completely inclosed the bud at the top and the buds were slightly larger.

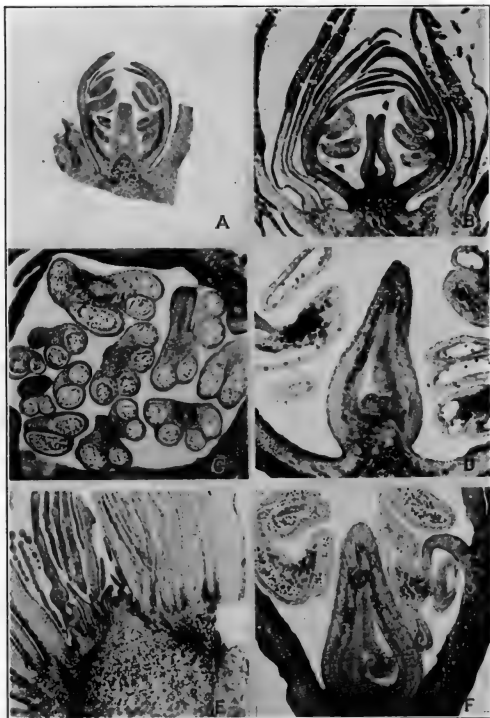
Buds collected January 1 showed a big increase in size of all parts. Ovule primordia were present as small knobs in the ovarian cavity, and the stigma had developed at the upper end of the lengthened style. A cross section of the ovary shows it not to be a perfect circle, but the walls fold in on one side, forming the future suture of the mature fruit. The ovules develop from the enfolded ends.

During January the buds continued to enlarge. The development in the ovary was slow. The stamens became larger and by February 6 had definite walls inclosing the large, rounded, loosely connected mother cells now ready for cell division (pl. 6). Material collected February 14 contained the four-celled or tetrad stage in pollen development. In apricots, as in pears, only one date showed this stage. By February 21 pollen was mature, but development of the ovule had progressed very little, as can be seen from Plate 5, D.

Following this stage, however, the development in the ovule progressed rapidly, and only five days later the two integuments and the nucellus were plainly evident in longitudinal sections of the ovary (pl. 5, F). The buds rapidly expanded, and the tree was in full bloom by March 2.

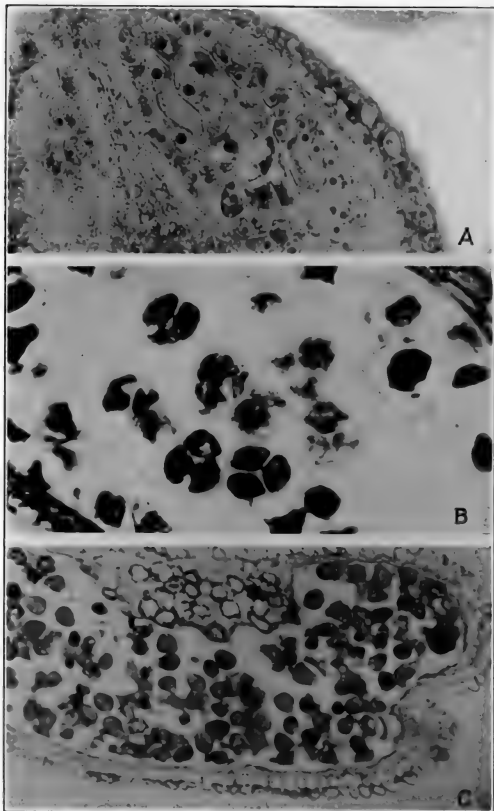
⁵ WALKER, R. M. THE FORMATION AND DEVELOPMENT OF THE FRUIT BUDS OF THE ROYAL APRICOT. 1917. [Unpublished thesis, Univ. Calif.]

⁶ HENDERSON, W. THE DIFFERENTIATION AND EARLY DEVELOPMENT OF THE FLOWER BUDS OF THE BARTLETT PEAR. 1916. [Unpublished thesis, Univ. Calif.]



Photomicrographs of the longitudinal sections through Royal-apricot fruit buds collected from university farm orchard, showing stages of development throughout the season of 1922-1923. (\times approximately 25 unless otherwise stated)

A, December 1, 1922; B, January 1, 1923; C, February 6 (cross-section through anther, showing pollen mother-cell stage); D, February 21 (ovule development in a cross-section of the ovary); E, February 25 (longitudinal section through leaf bud, \times approximately 30); F, February 26 (longitudinal section through ovary, showing further development of the ovule)



A.—Cross-section through a Royal-apricot anther, January 1, 1923, showing the pollen mother-cell stage. Note the large nuclei in the mother cells. (\times approximately 371)

B.—Cross-section through a Royal-apricot anther, February 14, 1923, showing the tetrad stage in the development of pollen. Each member of the tetrad ultimately becomes a pollen grain. (\times approximately 537)

C.—Mature pollen grains in cross-section of a Royal-apricot anther, February 21, 1923. (\times approximately 138)

DIFFERENTIATION AND DEVELOPMENT OF FRUIT BUDS OF THE PEAR GROWN UNDER THE VARIOUS CONDITIONS

HEAVY AND LIGHT PRUNING COMPARED

Up to July 20 buds from the heavily pruned and irrigated pear tree were slightly more developed than those from the lightly pruned and irrigated pear trees at the university farm (fig. 2). After that, however, the slight differences that existed fluctuated from one to the other, so that on the average they were uniform in development from August 1. As previously stated, these two trees did not show conditions as extreme as would have been desirable. The lightly pruned tree blossomed about two days earlier than the other. The blossoming of lightly pruned trees somewhat sooner than heavily pruned ones has been noticed in previous years also.

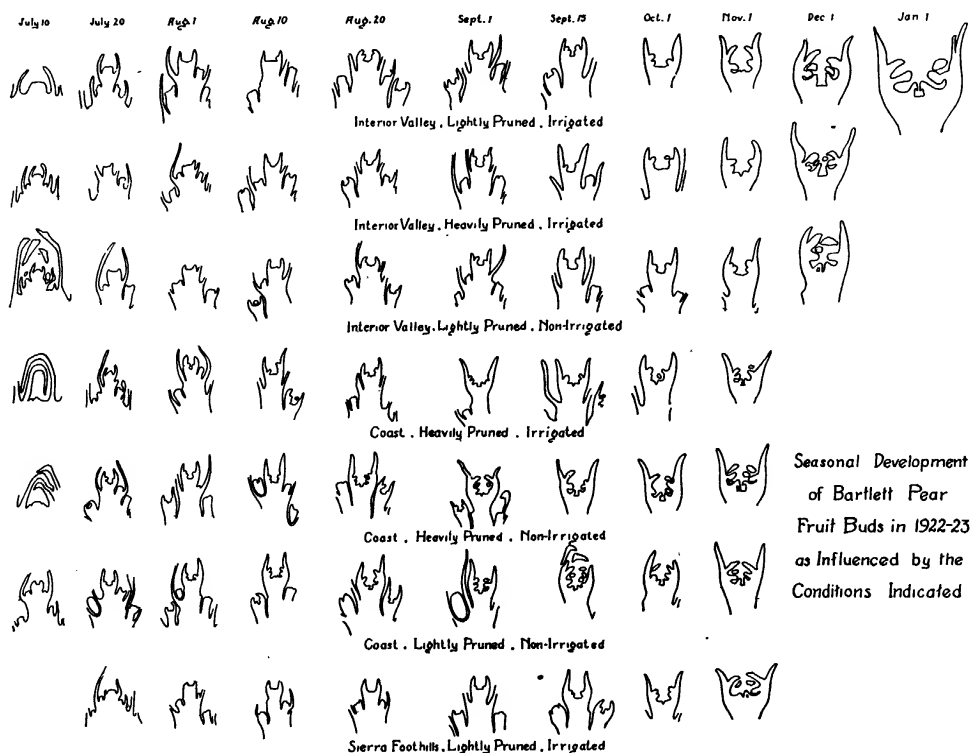


FIG. 2.—Outline drawings of longitudinal sections through Bartlett-pear fruit buds, showing the average stages of development on the various dates and under the various conditions

Although lightly and heavily pruned nonirrigated trees were observed in Sonoma County, the two trees did not grow near each other or on similar soil. However, when the comparison (fig. 2) was made between the two trees, it was found that the lightly pruned tree as compared with the heavily pruned one was in a more advanced stage of development when first examined (July 11) and kept slightly in the lead until August 21. At this date and afterwards the development was approximately the same under the two conditions.

IRRIGATION AND NONIRRIGATION COMPARED

On the university farm the fruit buds from the nonirrigated pear tree were farther advanced than those from the irrigated tree on July 7 and 20 (fig. 2). After the latter date, however, slightly the reverse

was the case. Both the heavily and lightly pruned and irrigated trees were slightly ahead of the nonirrigated tree on November 1, but there was no apparent difference December 1.

In Sonoma County the buds from the nonirrigated tree seemed to have a lead over those from the irrigated one throughout the season; both trees were heavily pruned (fig. 2). The lead was slight on July 11, but by September the buds from the nonirrigated tree were about 30 days in advance. These buds were noticeably ahead when the last samples were taken. Buds grown with the combined influence of nonirrigation and light pruning were slightly farther ahead of the heavily pruned and irrigated than of the heavily pruned and non-irrigated.

REGIONS COMPARED

Comparison of pear buds taken from Eldorado (Sierra-foothill conditions) and Yolo Counties (interior-valley conditions) under the same cultural treatment (fig. 2) showed that the buds grown under foothill conditions were, on July 20, slightly behind those from the interior valley and developed at a slower rate until the middle of August. Later collections, however, showed a rapid development in the buds from Eldorado County, which were leading October 1. By November these buds had reached a stage of development that buds grown under interior-valley conditions required from two to four additional weeks to reach.

Although pear fruit buds in the coastal valley and in the foothills apparently differentiated at about the same date, those in the cooler and more humid section developed more rapidly than those at the high elevation. The buds from the coastal valley were at least 30 days ahead by the middle of August. However, the buds grown under foothill conditions made such advance during September that the stages of development were equal October 1 and the Eldorado buds even slightly in the lead by November 1.

DIFFERENTIATION AND DEVELOPMENT OF FRUIT BUDS OF THE APRICOT GROWN UNDER THE VARIOUS CONDITIONS

LIGHT AND HEAVY PRUNING COMPARED

Pruning and irrigation treatments of apricot trees could not be compared in respect to fruit-bud formation, except at the university farm. The lightly and heavily pruned trees were irrigated. Both showed the initial signs of fruit-bud formation about August 10, and buds from each developed equally until the middle of September (fig. 3). Collections made September 15 and October 1 indicated the heavily pruned tree to be considerably ahead in floral development. This lead was not maintained, however, and the lightly pruned tree had buds showing the ovule primordia December 1, while none were found in buds taken from the heavily pruned trees.

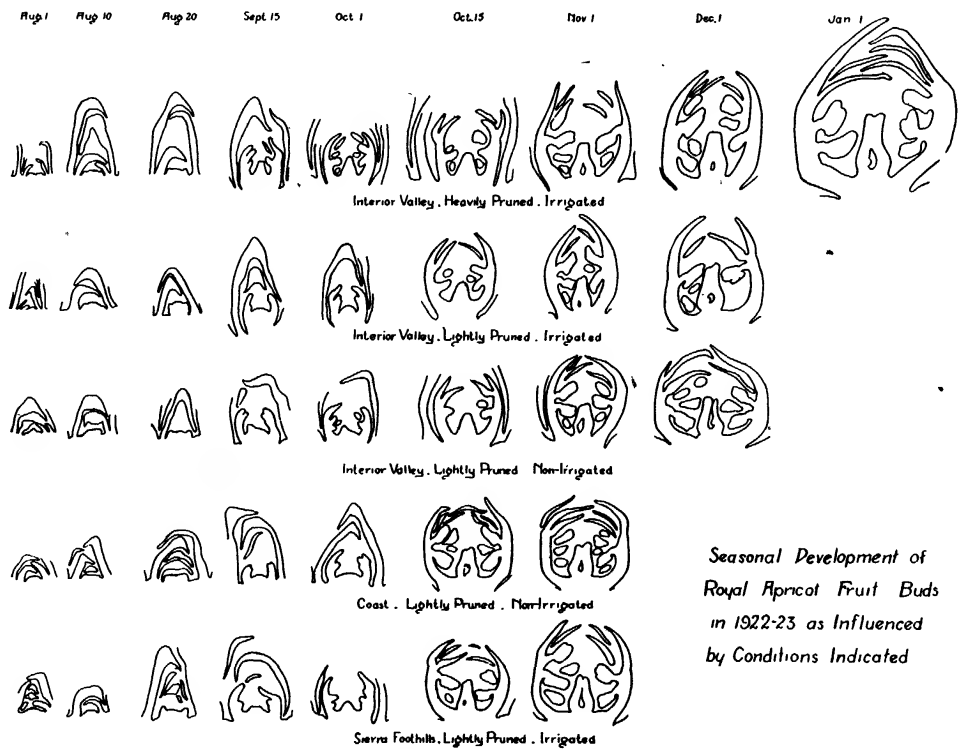
IRRIGATION AND NONIRRIGATION COMPARED

Fruit buds taken September 15 from the nonirrigated apricot tree in the university orchard showed a slight lead over those from irrigated trees, and they maintained it as long as collections were made (fig. 3). Prior to September 15, no difference in degree of development was observed.

REGIONS COMPARED

Buds taken August 1 from the tree in the coastal-valley section had smooth crowns, while no signs of this condition were evident in either of the other locations. However, in the subsequent collections the coastal-valley apricots seemed to be slightly behind, especially in pistil development, until the collections of October 15 (fig. 3). Buds collected from the coastal-valley district at this latter date showed a stage of development which buds from the other two locations required an additional month to reach. These more advanced buds contained well-defined pollen mother cells on October 15.

The apricots grown in the foothills and in the interior valley showed differentiation of fruit buds on August 10, which developed at about the same rate until in September, when the buds grown at the higher



Seasonal Development of
Royal Apricot Fruit Buds
in 1922-23 as Influenced
by Conditions Indicated

FIG. 3.—Outline drawings of longitudinal sections through Royal-apricot fruit buds, showing the average stages of development at the various dates and under the various conditions

altitude assumed a slight lead over the buds grown in the valley. At this time the buds under foothill conditions had anthers in the mother-cell stage of development.

DISCUSSION

With the long growing season in California it would seem logical to suppose that fruit-bud differentiation would take place earlier than in the East. This assumption appears well founded, since the Kieffer in Virginia (3) and the Wilder Early in Wisconsin (6) were found to show signs of fruit-bud differentiation about three weeks later than the Bartlett in California. Disregarding varietal differences, the late differentiation of pear fruit buds in Virginia and Wisconsin, as compared with the early fruit-bud formation in Cali-

fornia, might be explained by the relatively early beginning of the growing season in the latter State. Whether this or something else is the right explanation, it is clear that there are differences between localities. The writer knows of no data on the time of fruit-bud differentiation of the apricot except in California.

The development of fruit buds under interior-valley conditions is characterized by a slow but steady development of the floral parts through the summer and winter, with a rapid growth, of ovules especially, in the spring just before blossoming. Winter seems not to have any appreciable checking influence, although buds in California, even though blossoming earlier, are at a less advanced stage in December than are buds at the same time in the Eastern States.

Apricot buds differentiate a month or more later than pear buds, but develop at a much more rapid rate, and come into blossom earlier.

It would seem from the data collected that the differentiation both of the pear and apricot fruit buds, respectively, takes place at approximately the same time in all regions from which samples were taken. It is interesting, however, to note the more rapid development under coastal-valley influences. Development of fruit buds from the foothill sections, though slow at first, was only slightly less rapid than under coastal-valley conditions, while the stages of development of buds collected under interior-valley conditions were the least advanced November 1. In the case of the pear fruit buds, the lead of those from near the coast was maintained from the start, and this lead increased steadily until in October when they were about a month ahead of those grown at the higher altitudes. Development of fruit buds under foothill conditions was almost at a standstill following differentiation until September, when it progressed rapidly, due perhaps to the cooler weather there.

Gardner, Bradford, and Hooker (5) state that the rather restricted period of fruit-bud formation can be varied somewhat by cultural treatment, including, perhaps, any practice that modifies the rate of growth. As summer pruning has an influence on the time of fruit-bud differentiation (4), and since light and heavy pruning, popularly known as "long" and "short" pruning, cause very different reactions in other respects, why, then, should not light and heavy dormant pruning operate differently in respect to fruit-bud differentiation? Since heavy pruning induces much shoot growth, one might expect it to cause growth later in the season and thereby bring about later differentiation. This was found to be rather strikingly the case with the two pear trees under coastal influences. These trees grew on two types of soils, which may have caused the difference. When pruning methods were compared in the interior valley, the heavily pruned pear tree differentiated fruit buds a few days in advance, the reverse of the observation at the other location; however, the lightly pruned tree developed somewhat faster through July and August. With the exception of the collections made September 15 and October 1, the heavily and lightly pruned apricot trees on the university farm showed the same rate of development. It would seem from the data available, at least in the case of the pear, that light pruning as practiced in California tends to hasten development.

Contrary to Goff's findings (7), and coinciding well with Kirby's conclusions (9), the lack of moisture apparently tends to hasten fruit-bud differentiation and subsequent development. The retarding

influence of irrigation on fruit-bud development of the pear was most strikingly shown under coastal-valley conditions. Only buds collected July 7 and 21 from nonirrigated trees on the university farm showed any lead over those taken from irrigated trees. No difference in development of fruit buds of irrigated and nonirrigated apricot trees under interior-valley conditions could be observed until September 15. At this date the buds from the nonirrigated trees assumed a lead which they maintained as long as collections were made.

SUMMARY AND CONCLUSIONS

A study was made of the influence of certain cultural and environmental factors on the differentiation and development of Bartlett pear and Royal apricot fruit buds. In order to study the environmental influences upon fruit-bud formation and development, material was collected at short intervals between July 7 and November 1, 1922, and from three typical fruit-growing regions of California, namely, Sierra foothills (Eldorado County), interior valley (Yolo County), and coastal valley (Sonoma County). In addition, samples under interior-valley conditions were collected until blossoming time. To study the effect certain cultural practices might have, lightly pruned trees were compared with heavily pruned ones, and irrigated were compared with nonirrigated trees in respect to time of fruit-bud formation and the rate of development. The following conclusions seem justified:

1. Pear fruit buds begin to differentiate at approximately the same date under coastal-valley, interior-valley, and foothill conditions.
2. Apricot fruit buds begin to differentiate at approximately the same date under coastal-valley, interior-valley, and foothill conditions.
3. The high altitude of the foothills seems to have a retarding influence on fruit-bud development until the middle of September when development becomes more rapid.
4. The humid coastal conditions apparently stimulate rapid development of pear buds after differentiation. This is not the case with apricots until October when development becomes extremely rapid and the buds go into the winter at a more advanced stage than is found under either interior-valley or foothill conditions.
5. The dry, hot, interior valley seems to induce a steady uniform development of both pear and apricot fruit buds; however, these have not reached the advanced stage of development by early winter that buds from the coastal valley and foothills have attained.
6. The inception of fruit-bud differentiation seemingly is not influenced to any extent by either heavy or light dormant pruning. Light pruning perhaps tends to induce a slightly more rapid development for six to eight weeks following fruit-bud differentiation of the pear.
7. Irrigation seems to have a retarding influence on fruit-bud differentiation and development.
8. Environmental conditions during winter, as found in the principal fruit-growing districts of California, do not seem to have any checking influence on fruit-bud development of the pear and apricot.

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BENZENE AS A LARVICIDE FOR SCREW WORMS¹

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INTRODUCTION

The larval stage of *Cochliomyia macellaria* Fab., generally known among stock raisers in the Southwest as the screw worm, causes considerable loss to the livestock industry, estimated as high as \$5,000,000 in some years. It has been apparent that the larvicides used to kill the worms are either toxic to the animal or at least in most cases detrimental to the healing of the wounds. This toxicity was at first attributed to the screw worm, but as many cases were observed where the animal was practically consumed by the larvae and still lived until the loss of blood or injury to some vital organ brought death, it was surmised that the treatments with larvicides were the cause of many deaths. During the summer of 1916 systematic work was begun to find a more efficient larvicide than the phenols and chloroform which were generally used.

At first an attempt was made to add something to these larvicides to counteract the toxic properties. As this was not successful it was deemed best to look for a chemical that might be used with more satisfactory results. Several chemical groups were studied for possible larvicides.

EXPERIMENTAL PROCEDURE

All available chemicals with possible larvicidal value were selected for laboratory tests to determine whether they would kill the larvae of the screw-worm fly. The first tests were made by pouring the chemical on a number of larvae in a tube, or dusting on just enough to cover the larvae. All chemicals that stood this test were selected for tests on infested rabbit carcasses; and from these, after the elimination of the ones deemed too toxic at the greatest dilutions to be used on wounds, the others were selected for field tests on living animals.

CHEMICALS USED IN FIELD TESTS

Ethyl ether, benzene, toluene, xylene, cymene, carbolic acid, safrol, eugenol, ortho-, meta-, and para-cresol, nicotine, pyridine, nitrobenzene, methyl chloride, chloroform, carbon tetrachloride, methyl alcohol, amyl alcohol, formaldehyde, picric acid, formic acid, carbon disulphide, sodium cyanide, mercurous chloride, mercuric chloride, borax, sodium arsenate, antimony chloride, benzine, gasoline, kero-

¹ Received for publication February 21, 1925; issued January, 1925.

sene, fuel oil, crude petroleum, coal-tar dips, creosotes, and turpentine were used in field tests.

These substances were first tested in very minute quantities on laboratory animals, many being diluted with light paraffine oil or water. All that proved markedly toxic or irritating to the animal as the amounts used increased to a point that would kill the screw worms were eliminated. The remaining ones were then tested, at first on small wounds, and, when no marked toxicity or irritation was observed, they were used on still larger wounds. The elimination process continued until only ethyl ether, benzene, toluene, xylene, chloroform, and carbon tetrachloride were used.

Ethyl ether was considered too volatile for field use, and was eliminated early for this reason. Toluene and xylene were about as effective as benzene but were eliminated because they were less available than benzene. Chloroform, chemically pure (not decomposed), was found to be quite satisfactory. Samples other than the chemically pure product, however, were found to be objectionable for the same reasons as held for the decomposed chloroform. Carbon tetrachloride and the chemically pure chloroform have a laxative effect on the wound that is undesirable.

BENZENE AS A LARVICIDE

Benzene has the same rank as a larvicide for the screw worm as ethyl ether, chloroform, carbon tetrachloride, and xylene; the larvae in each case, when moistened with these materials, became inactive in an average of 40 seconds. In dry wounds, the time required to kill the larvae is practically the same for each and is about the same as that for the larvae moistened with the chemicals. Where the wounds do not have good drainage, the larvae are killed very slowly with benzene, ethyl ether, and xylene, as these materials float on the blood and serum, and it has been found that the larvae can live submerged in the wound from 20 to 30 minutes. Chloroform and carbon tetrachloride sink into the blood and serum and mix with it, and all larvae are killed nearly as quickly as in a dry wound. To kill the larvae quickly with benzene in such wounds it is necessary to dry the wound first with cotton or a cloth and to apply the benzene immediately.

EFFECT OF BENZENE ON WOUND

Most wounds infested with screw worms have a considerable flow of fresh blood and serum. As long as the wound is dry it is not so attractive to the flies, and for this reason any chemical that has a tendency to laxate the wound is not desirable. Benzene acts as a styptic, and the blood flow stops in most cases as soon as all of the larvae are killed. When benzene is applied, and the wound takes on a dry, pale appearance, which lasts from 5 to 10 minutes or more, a wound dressing and fly repellent which adheres well to all parts should be applied, making a perfect coating which should continue the styptic effect. In no case has any indication of injury to the tissues been observed after an application of benzene, and the wound usually heals soft and dry without scabbing or cracking. On clean, smooth wounds there is hardly ever any dead tissue after treatment.

TOXICITY OF BENZENE TO ANIMALS

No tests have been made to determine the toxicity of benzene to the higher animals except in its practical use as a larvicide for the screw worm. No toxic effect has been observed on sheep and goats where the wool and mohair were entirely saturated on all parts of the body, and wounds as large as 8 inches in diameter were treated with sufficient amounts to kill all larvae in them. Such animals have in all cases been healed when they were kept under observation, although in some instances they were down and unable to move when treatment was begun. Goats, sheep, and calves quite commonly have infestations of worms in the mouth to the extent that all of the teeth are lost. In treating these cases care has been used to prevent the animals from swallowing any of the benzene and it is applied in small amounts (2 to 5 c. c.). In no case has any ill effect on the animals been observed.

In the treatment of more than 3,000 cases of worms in cattle, sheep, goats, hogs, dogs, and chickens, there has never been any noticeable toxic effect on the animal. In the work on the range it has been remarkable and significant to note the number of animals reported "as good as dead" by the herders that recovered completely after treatment. Most ranchmen have had the idea that any serious infestation with screw worms would kill the animal and for this reason many animals badly infested with worms have been killed and burned. In 2,843 cases of infested animals treated with benzene under the writer's personal observation, only 5 have died. In 3 of these cases the worms had entered the body cavity or were present in the head of the animal and had affected the nervous system.

Holland² makes the following statement: "A narcotic effect is produced by accidental inhalation of benzene vapor in factories. One ounce (30 c. c.) taken by the stomach caused death after symptoms such as headache, giddiness, bluish flush of the face, delirium, convulsions, and coma." While a wound in a big steer was being treated a pint bottle was kicked by the steer and broken and the contents thrown into the writer's face. There was some difficulty in breathing for about two minutes. The excess was wiped away and the face and eyes had a numb, cool feeling for about five minutes; there was then a slight burning sensation in the eyes which lasted about ten or fifteen minutes, succeeded by a very pleasant, clean, sensation in the eyes. No other symptoms were noticed at the time or later. When benzene is spilled on clothing and penetrates to the skin there is a burning sensation, but to the writer's knowledge no injury has ever been observed.

BENZENE IN PRACTICAL USE

Besides the experimental work in which benzene has been used, approximately 5,000 animals or more have been treated by ranchmen and herdsmen. The benzene was furnished by the Bureau of Entomology and reports were made to the bureau. Fifty-three ranchmen have been furnished benzene in amounts of 1 pint to 1 gallon each. Reports have been received from all of these and some were quite

² HOLLAND, J. W. A TEXT-BOOK OF MEDICAL CHEMISTRY AND TOXICOLOGY. Ed. 5, rev., 683 p., illus. Philadelphia and London. 1917.

complete as to the efficiency of the material as a larvicide, its effect on wounds, and toxicity to animals. In no case has any indication of toxicity to the animals or ill effects on the wounds been reported. In a few instances the reports were to the effect that benzene was slow to kill the worms in some cases. These probably were cases in which the wounds were not well drained and were not cleaned before benzene was applied. Most of the men feel that it is a very efficient larvicide and, on account of the effect on wound and animal, prefer it to any other they have ever used.

AVAILABILITY AND PRICE

Benzene used in the work has been of the grades known in the trade as "commercially pure" and "90 per cent." These grades are available to the trade in amounts that will be far beyond the demand for larvicidal purposes, and the source is practically inexhaustible. Commercially pure benzene is usually about 2 cents per gallon cheaper than the 90-per cent in quantity lots. Quotations on the commercially pure benzene, for June, 1924, in tank lots, were as low as 21.5 cents per gallon; in drums, 28 cents per gallon; these prices at production centers.

CONCLUSIONS

Benzene is an effective larvicide for the screw worm, when used properly. It has no ill effects on the wounds, and acts as a styptic temporarily to make the wound less attractive to the adult flies. When used in wounds as a larvicide for the screw worm it has not been found to be toxic to any animal.

METHODS OF STIMULATING GERMINATION OF WESTERN WHITE-PINE SEED¹

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INTRODUCTION

In forestry as in agriculture seeds are often encountered which are difficult to germinate and are called "rebellious." In this class belong the seeds of many hardwood species and not a few conifers. The soft or white pines are much noted for this characteristic, *Pinus monticola* in particular.

Prompt germination of western white-pine seed has constituted a real problem in the Forest Service nurseries of the northern Rocky Mountain region. In one instance 35 beds spring-sown with 5 pounds of seed per bed gave a meager and irregular germination the first summer and from 3,000 to 40,000 seedlings per bed the second season. Such results add greatly to the expense of raising nursery stock, in that the beds require care for two years instead of one and occupy double the usual space.

When this problem was taken up by the field station of the Priest River Forest Experiment Station, in northern Idaho, it was assumed as a working basis that delayed germination was due to inherent physiological characteristics requiring after-ripening of the seed or a period of rest,² or to the impermeability of the seed coat to water from without and to the root tip from within. If the first hypothesis were true, some process of stimulation by stratification or by chemical or physical means might be effective. If the latter were the case, reduction of the seed coat by chemical or mechanical means would be likely to bring success.

German investigators, among them E. Zederbauer,³ who has done considerable work with conifer seeds, maintain that there is a relation between the time of ripening and of germination, and that the seed which is shed in the autumn germinates slowly while that which is shed in the spring germinates more rapidly. Zederbauer places *Abies alba*, *Pinus cembra*, and *Pinus strobus* in the slow class. The seed of these fall in the autumn. *Pinus sylvestris*, *Pinus nigra*, *Picea excelsa*, and *Larix europea* he places in the rapidly germinating class. The seed of this latter class is shed in March, or, as with *Picea excelsa*, in February. The seed which remains on the ground during the winter is subjected to the influences of water and frost, differences in ground conditions and other factors, all of which may produce chemico-physiological changes.

Early work on this project at the Priest River Forest Experiment Station embraced treatment with sulphuric acid and other chemicals.

¹ Received for publication Dec. 22, 1924; issued January, 1925.

² COULTER, J. M., BARNES, C. R., and COWLES, H. C. A TEXTBOOK OF BOTANY FOR COLLEGES AND UNIVERSITIES. v. 2, p. 931-932, illus. [1911.]

³ ZEDERBAUER, E. DIE KEIMPRÜFUNGSDAUER EINIGER KONIFEREN. Centbl. Gesam. Forstw. 32: 306-315, 1906.

Tests were also made in different media and germinators, but with poor results. The invariably low germination obtained in all of the tests, even over long periods up to 200 days, led to the belief that the quality of the seed itself might be impaired either by storage or extraction. As soon, therefore, as new seed could be collected it was extracted from the cones under low temperatures and stored in air-tight containers. A complete series of new experiments, covering more than 300 different tests, was then outlined. These embraced stratification in different media for various lengths of time, with or without heat or cold treatment, soaking for different periods of time in acids and various chemicals, heating and freezing the seed, abrasion of the seed coat by cutting or other mechanical treatment, and studies on the need of after-ripening and necessity for a period of rest.

STRATIFICATION

Stratification of the seed as a preliminary treatment was suggested at the outset, for in this manner the seed would receive a treatment in bulk under conditions similar to those in the seed bed but without sowing and carrying the bed for two seasons.

The seed was laid 5 inches deep in 10-inch wooden boxes, with tops and bottoms of wire mesh to protect from mice and admit heat, cold, moisture, and provide drainage. Sand, sawdust, clay loam, and duff from the forest floor were used in parallel boxes. One series of these was sunk to the upper level of the box in a sandy bench, another in clay loam, a third on moist bottom land under young timber, and a fourth in clay loam in a cellar. Repeated installations were made March 5, June 6, and September 5, in order to determine the effect of the length of time kept in this condition. Sowings from all of these were made in the nursery early and late in the spring and autumn of the same year, and in the spring of the following year.

At the time of the first sowing, certain samples were given from 3 to 30 days additional treatment by heating the seed in moist greenhouse sand and from 3 to 9 days in moist cotton flannel in the incubator. In sand the temperature records show a variation from 70° to 100° F. and in the incubator from 80° to 102°.

When the first lots were removed from the stratification boxes, 37 days after being laid down, from 3.7 to 6.3 per cent of the seed had germinated, the lowest percentage being obtained from that sown in the clay in the cellar, and the highest in the clay on moist bottom land. Decay had also set in, but in no case was more than 1 per cent of the seeds affected, and the greatest amount of decay occurred with the highest germination. The germination continued during the heating in the sand and in the incubator and in some cases attained 1 per cent in 9 days, and in one case 8.6 per cent in the greenhouse sand in 30 days.

These seeds and also those taken directly from the stratification boxes were sowed in the nursery April 20, together with a check sowing. Three beds were used, one of which was open, another covered with a sphagnum-moss mulch, and a third covered with tar and felt paper. Triplicate samples representing each individual treatment were thus sowed in parallel series, one in each of these beds.

The great number of tests and the many mediocre results make it futile to give the results in detail. Only the best results from each location and medium are given (Table I).

TABLE I.—Maximum germination according to each location and medium for stratification

Stratification		Secondary treatment		Germination ^a
Location	Medium	Nature	Period	
			Days	Per cent.
Clay loam bench.....	Sandy.....	Greenhouse.....	9	19.5
Do.....	do.....	Incubator.....	9	19.5
Do.....	Clay loam.....	do.....	9	23.0
Do.....	Sawdust.....	do.....	3	39.0
Do.....	Duff.....	do.....	9	21.0
Loamy sand.....	Sandy.....	do.....	3	32.0
Wet bottom land.....	Clay loam.....	Greenhouse.....	6	39.0
Clay loam cellar.....	do.....	Incubator.....	9	14.5
Not stratified.....	Check.....	do.....	3	31.0
Do.....	do.....	Greenhouse.....	30	32.0
Do.....	do.....	No heating.....		21.0
Clay loam bench.....	Clay loam.....	Tips cut with knife.....		32.0
Not stratified.....	do.....	No heating; tips cut with knife.....		67.0

- ^a Seed bed covered with moss mulch, except as otherwise noted.
- ^b Seed bed uncovered.
- ^c Seed bed covered with tar paper.

Though showing a slight increase in some cases over the check tests, this method of stratification failed to give satisfactory results. Thirty days' simple treatment in the warm moist sand in the greenhouse or three days' moist heating in the incubator gave almost as good returns as did any of the stratified samples.

Germination by autumn sowing of the earliest as well as the later stratified seed, and also germination from the spring sowing of all of these the following year, were much below the percentages in Table I, even below the earlier check tests. Failure was shown by careful cutting tests to be due to rotting of the seed.

Germination of seed placed in sawdust was slightly better in most cases than from the other media, and the later tests show that the seed may remain unimpaired longest in sawdust. Bark-free sawdust was used in order to avoid the possibly injurious effect of tannin.

The tests bring out very clearly the beneficial effect of a moss mulch in aiding germination, for in more than 90 per cent of the cases the highest tests occurred in the mulched bed; and this method of preserving moisture of both air and soil and of equalizing the temperature promises better germination in seed beds in Montana and Idaho, where the atmospheric humidity is lower and the diurnal fluctuations in temperature are greater than in the Central or Eastern States.

CHEMICAL TREATMENT

The treatments designed to hasten germination through corrosion of the seed coat by chemicals included soaking for periods of 8, 15, 25, 35, and 45 minutes in concentrated solutions of sulphuric, acetic, and citric acids. One of the samples was heated by the addition of water to the sulphuric acid. Some were treated for 15, 30, 60, 120, and 180 minutes in saturated potassium permanganate and potassium sulphate, and others for 5, 10, 20, 30, and 60 minutes in carbolic acid. Each of the samples chemically treated was washed in 12 waters to prevent further absorption and chemical injury to the seed kernel.

Samples of seed were frozen in an ice-cream freezer for 6, 12, 18, and 24 hours after having been soaked in cold water to test the effect of the mechanical action of freezing. One sample was soaked in full-strength sulphuric acid 15 minutes, thoroughly washed, and frozen for 24 hours; another was frozen 24 hours and then heated in the incubator for 3 days.

The results of these treatments and of the several check tests, as well as of samples with holes cut in the seed coat, are given in Table II:

TABLE II.—Highest germinations obtained by soaking, freezing, and chemical treatments

No.	Treatment	Time	Germination in seed bed (250 days)			
			Uncov- ered	Moss mulch	Check tests	
					Uncov- ered	Moss mulch
			Per cent	Per cent	Per cent	Per cent
1	Sulphuric acid.....	45 minutes.....	38.5	28.5	4.5	3.5
2	Sulphuric acid, then heated to 144° by water and left standing 30 minutes.	do.....	40.5	30.0	6.0	5.5
3	Acetic acid.....	40 minutes.....	7.0	14.0	5.5	10.0
4	Citric acid.....	120 minutes.....	7.0	5.5	8.5	2.5
5	Sulphuric acid.....	15 minutes.....	11.0	26.5	7.0	10.0
6	Freezer.....	24 hours.....				
7	Incubator.....	3 days.....				
8	Potassium permanganate.....	180 minutes.....	19.5	19.5	7.5	7.5
9	Potassium sulphate.....	120 minutes.....	26.5	24.5	5.5	4.0
10	Carbolic acid.....	30 minutes.....	15.0	12.5	5.0	4.0
11	Tips worn by rubbing on file.....		34.0	21.0		
12	Tips cut with knife.....		51.0	56.0		
13	Seed coat cracked by crushing.....		0.0	1.0		
14	Seed coat split open with knife.....		41.0	29.0		
15	Hole cut in seed coat, leaf end.....		40.0	22.0		
16	Soaked in sulphuric acid until seed coat could be rubbed off.....		24.0	42.0		
17	Seed coat entirely removed.....		43.0	4.0		

In the tests with chemicals the highest nursery germination of 38.5 per cent was obtained by soaking the seed in concentrated sulphuric acid for 45 minutes. The addition of water to produce heat raised this to only 40.5 per cent and is not considered a sufficient gain to be worth while. The treatments with the other chemicals resulted, on the whole, in very little stimulation. The soaking in acid 15 minutes with later freezing and heat treatment produced germination no better than soaking in sulphuric acid 35 minutes without freezing. Freezing as well as heating by these methods would therefore seem to be useless operations.

Not only a greater total germination but a more rapid rate than in the sulphuric acid tests was obtained by the mechanical treatments, especially the cutting of a very small hole in the end of the seed with a knife. (See fig. 1.) Mechanical abrasion of the seed coat, if it can be done without injury to the kernel, is therefore sure to give a higher and more rapid germination than either stratification or chemical corrosion of the seed coat.

Preliminary treatment to stimulate the germination of white pine by soaking in sulphuric acid is a practical and efficient method which

may be used on a large scale for spring sowing at the nursery. It requires only a zinc-lined vat as acid container, running water, and paraffined hardware cloth baskets for dipping and rinsing the seed. After the treatment the acid is somewhat colored but has not lost its value for sterilizing purposes. Dry seed only must be used for the acid treatment, for experiments have shown that wet or soaked seed are injured by the acid.

These tests, particularly those in which the seed coat was removed, show beyond doubt that delayed germination in the seed bed the first season after collection is due entirely to the obstruction of the seed coat itself and not to any inherent physiological character of

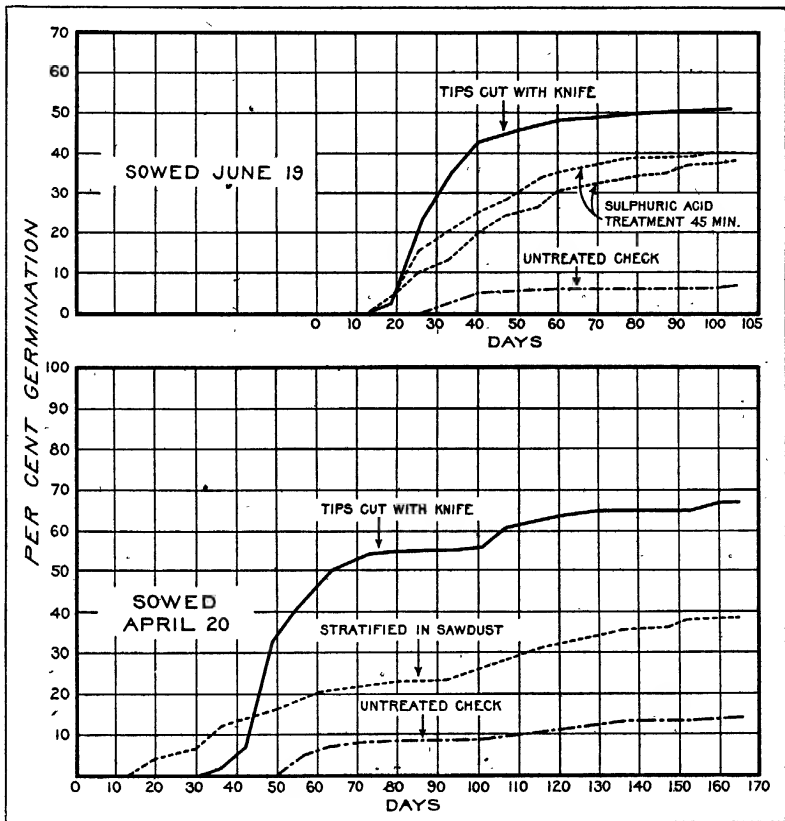


FIG. 1.—Nursery germination of western white pine

the seed. After-ripening or the need for a period of rest may still be factors to contend with, but at any rate these do not hinder germination during the spring and summer following seed production.

MECHANICAL TREATMENTS

On the strength of the excellent results obtained from the cutting or removal of the seed coat, experiments were undertaken to devise a churning apparatus coated with emery or carborundum dust for the reduction of the seed coat. At this time, however, T. R. Truax had produced such a machine by which red clover germination was materially hastened. Accordingly, several samples of western white-pine seed were sent for treatment.

Tests of the earliest samples by this treatment gave negative results, because most of the seeds were broken or crushed, but in the second tests 16.5 per cent germination was obtained in 40 days compared with only 11.3 per cent in the checks. Parallel samples with tips cut gave 20 per cent in the same period. But though germination was hastened by this means, the seed thus treated eventually gave a lower test than the untreated because of mechanical injury.

This shows that it may be entirely feasible to increase the rate of germination by scarification, provided suitable machinery is available. Though the perfection of such machinery is beyond the province of the present project, a modified type of barley pearling machine is suggested as suitable for this purpose. In this machine the grain is scarified between a perforated outside steel casting and an inside revolving carborundum wheel. For white-pine seed it would be necessary to substitute a fleece-lined sheepskin for the steel casting so as not to crush the seed. The process of scarifying white-pine seed, however, is more complicated than rice or barley, because very little pressure can be used to force the seed against the abrading surface, and because the resin will clog the machine very quickly.

A series of treatments with coarse and fine needles was tried later, the chief purpose being to determine whether or not germination could be hastened by blowing the seed against a bank of needles. No machine was made, but the seeds were punctured by the needles as they lay on the blotter. The needles were prevented from penetrating deeper than through the seed coat by surrounding the tip with sealing wax. The bank of needles was made by setting them in a piece of paper similar to pins stuck in a folded strip of paper and rolled compactly and solidified by sealing wax. This bank made 32 points of needles within a circle one-fourth inch in diameter.

By this treatment the seed was injured, presumably by mechanical impact, for only one sample of those treated showed any germination, and this only 5 per cent in 40 days.

EXPOSURE TO LOW TEMPERATURES

Additional experiments in soaking and freezing the seed were made during the winter of 1916 in order to try the mechanical effect of the water in freezing and thawing and of exposure to low temperatures for a longer period in air and snow. This work was undertaken largely on the strength of the excellent results recorded by Pittauer⁴ in stimulating germination of *Pinus strobus*. With dry freezing and 24 hours' soaking after the freezing, he obtained 75 per cent germination in 28 days, and with snow freezing he obtained 56 per cent, with only 17 and 18 per cent for the untreated samples.

The Priest River experiments, covered in Table III embraced 10 separate groups of the same seed which were divided into three main divisions as follows: Groups A to D exposed to freezing in the air, some soaked and others not; Groups E to H, soaked and unsoaked, frozen in the snow; and Group I, untreated check.

⁴ PITTAUER, G. ÜBER DEN EINFLUSS VERSCHIEDENER BELICHTUNG UND EXTREMER TEMPERATUREN AUF DEN VERLAUF DER KEIMUNG FORSTLICHEN SAATGUTES. Centbl. Gesam. Forstw. 38: 157-172, 213-224, illus. 1912.

TABLE III.—Results of freezing in air and snow, with and without soaking in water

Group No.	No.	Treatment	Percent- age of germi- nation in 50 days
A	1	Freezing in air, not soaked	3.5
	2		4.0
	3	Tips cut	3.0
B	4	Freezing in air, all soaked before freezing	17.5
	5		6.5
	6	Tips cut	10.2
C	7	Freezing in air, soaked after freezing	6.5
	8		7.5
	9	Tips cut	11.5
D	10	Freezing in air, soaked before and after freezing	15.5
	11		11.0
	12	Tips cut	5.0
E	13	Freezing in snow, not soaked	5.5
	14		8.5
	15	Tips cut	6.5
F	16	Freezing in snow, soaked before freezing	6.0
	17		4.5
	18	Tips cut	5.5
G	19	Freezing in snow, soaked after freezing	5.5
	20		10.0
	21	Tips cut	6.5
H	22	Freezing in snow, soaked before and after freezing	7.5
	23		5.5
	24	Check tests, not frozen, not soaked	6.5
I	25	Tips cut	15.0

The soaked seed was immersed in cold water for 24 hours, and all of the samples except those used for checks were then exposed for 40 days, from February 22 to April 3, and tested in sand in the greenhouse. While complete greenhouse germination of this species requires 200 days, and is usually at a maximum after 100 days, these tests were concluded after 50 days in order to bring out more clearly the stimulating effect. A record of the daily temperatures during exposure of the seed is given below.

Temperature	Feb. 22-29	Mar. 1-15	Mar. 16- Apr. 3
Mean maximum	37	35	41
Minimum	21	25	28
High	41	44	48
Low	14	6	22

In Groups A to D, the highest germination (17.5 per cent) resulted from soaking 24 hours followed by air freezing; the next highest germination was 15.5 per cent, resulting from air freezing and soaking both before and after freezing; only 7.5 per cent germination resulted from soaking after air freezing; and germination in the check tests was from 5.5 per cent to 6.5 per cent. This indicates that germination may be hastened by this means and that soaking before freezing is more influential than soaking afterwards.

Better results were also obtained by soaking before freezing when snow was used, but germination was not so high as by air freezing. There was no noticeable difference between those not treated and those soaked after freezing.

Since the best results in this series of experiments exceeded those with the tips cut, which in previous tests had given the highest

germination, it is evident that a method is indicated by which large amounts of seed may be treated in bulk with very little labor and expense. After exposure the seed should promptly be mixed with moist bark-free sawdust and kept until the time of sowing, under temperatures between 35° and 40° F. Molding may be prevented effectively by stirring seed and sawdust every other day. If, after treatment, this seed should be allowed to hang or lie in storage until the time of sowing, it would most certainly deteriorate very rapidly, either by molding or by drying.

It should be noted that these results conform to those obtained by Pittauer, in that better germination was obtained by air than by snow freezing.

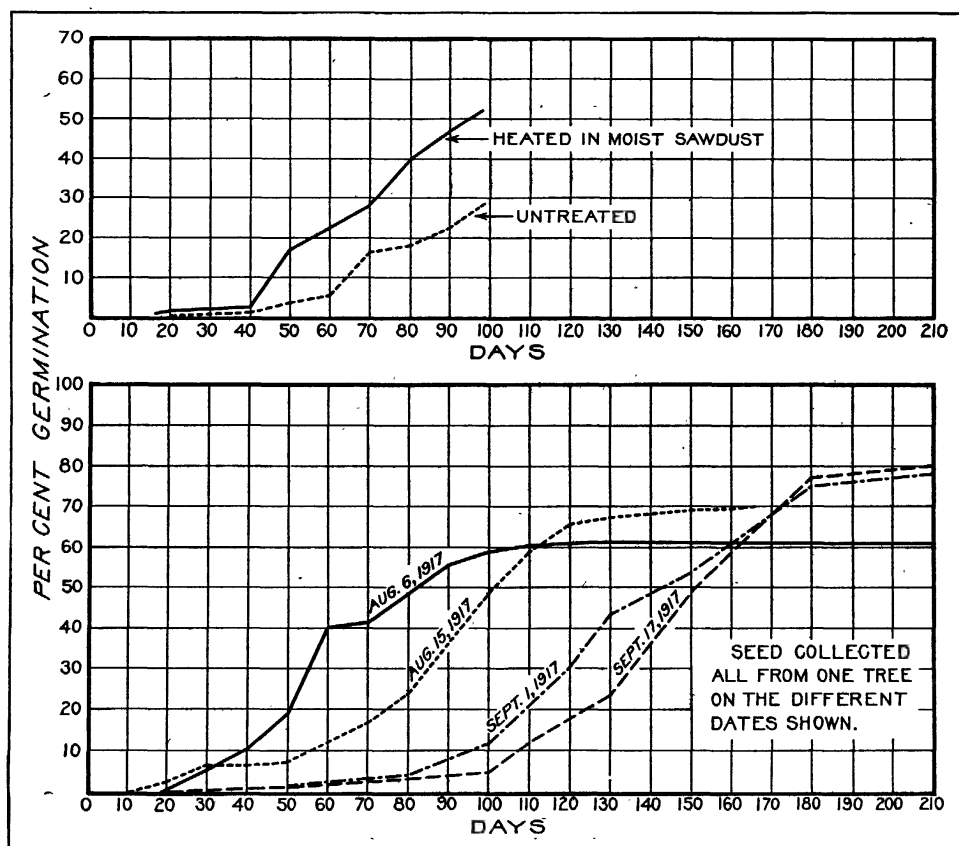


FIG. 2.—Greenhouse germination of western white pine seed, showing rate of germination during entire period of tests

PRELIMINARY TREATMENT IN MOIST SAWDUST

During the winter of 1916-17 a few samples of the western white pine seed were mixed with moist sawdust in a small wooden box and kept warm in the greenhouse. The seed was then covered with moist cotton and stirred thoroughly every day to prevent molding. This method, recommended by Johannes Rafn, has been in use for some time at the Danish seed control station in Copenhagen. The results of this treatment are given in Table IV and are shown graphically in Figure 2.

It is evident that germination may be nearly doubled by this means and that a three weeks' preliminary treatment is as good as or better than a longer or shorter time. This inexpensive method may

TABLE IV.—Preliminary treatment in moist sawdust

Manner and length of treatment		Germination in each period	
Water	Moist sawdust	50 days	100 days
Hours	Days	Per cent	Per cent
24	13	5.2	31.0
24	23	17.0	53.5
24	32	8.0	47.0
Untreated.	-----	2.8	29.5

then be recommended both for greenhouse sowing in sand and various germinators and for spring sowing in the nursery. It is necessary to maintain uniform medium moisture conditions by keeping the seed covered to avoid extremes of temperature, and by daily stirring to prevent development of mold spores. The soil temperature in these tests fluctuated between 60° and 90° F.

AFTER-RIPENING AND PERIOD OF REST

The investigations have revealed several methods by which white-pine germination may be hastened, but the question still remains unanswered whether the seed requires a period for after-ripening or for rest before active germination can take place. In order to throw light on this problem fresh seed was collected from a squirrel cache in a 250-year-old pine stand September 4, immediately extracted, and sowed soon afterwards, both in sand in the greenhouse and in the nursery. This seed had all the appearance of ripeness and was extracted at once without artificial heat.

Greenhouse sowing took place November 20. The sowing was of duplicate samples of 500 untreated seed and of seed with the tips cut as in the previous nursery tests. Of these the sample with the tips cut began to germinate December 7, or 17 days after sowing, and continued until complete with 74.6 per cent on April 20 following. The untreated sample began to germinate December 13 and was complete with 79.2 per cent July 10 following. Those untreated and sown in the nursery germinated very rapidly soon after warm weather developed, one lot reaching 79 per cent germination before June 19 and the other 83 per cent.

The prompt and complete germination of this seed in the greenhouse proved without doubt that after-ripening for this seed is of small moment. It did lead, however, to the belief that the seed was not entirely ripe. In order to prove this point further, seed was collected at successive intervals during the next good seed crop and tested.

The seed for this experiment was collected at intervals of about two weeks, from a well-developed tree about 75 years old, was extracted under ordinary September and October temperatures indoors without stove or other heat, and sowed in triplicate samples in sand in the greenhouse November 21. The results (given in Table V and fig. 2) are very interesting in showing a consistent and more rapid germination for the earlier lots, but in every case a higher final test for the seed of later collection.

CEREAL INVESTIGATIONS

TABLE V.—*Maturity test: Germination according to time of ripening*

Collection date	Greenhouse germination				Weight of 1,000 clean seeds
	50 days	100 days	150 days	210 days	
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Grams</i>
Aug. 6.....	18.8	58.7	61.2	61.2	11.40
Aug. 15.....	7.0	48.7	69.2	70.7	13.35
Sept. 1.....	1.7	11.7	58.8	78.3	18.50
Sept. 17*.....	1.3	5.0	49.0	80.0	17.35

* Seed falling after this date.

This more rapid germination rate of the earlier seed may have been caused by immature seed coat and the greater total germination of the later collection by a greater number of seed attaining ripeness.

When it is now considered that the sample in this maturity test collected September 1 gave a final germination of 78.3 per cent, which was as high as that in the after-ripening test collected September 4, there seems little ground for supposing that the seed was unripe or green except perhaps as regards the seed coat.

Sowing of fresh seed in the autumn will not always bring prompt spring germination, for if much rainy winter weather occurs, with light snow and temperatures of from 32° and 50° F., the seed will be injured.

Observations on the character of cones and seed showed that on on August 6 the cones were purple and the seed light brown, not filled out, and somewhat milky. On August 15 the cones were still purple and the seed light-colored but firmer and better filled. On September 1 there were also many light-colored seed, but all were filled out as well as those of September 17. The appearance of the seed and the weight on September 1 indicate that it was ripe at this date.

A microscopic examination was made of the structure and composition of the tegumentary system of the seed collected August 6 and September 17 from the same tree for the purpose of finding out whether the differences in time and amount of germination were due to greater development or hardening of the seed coat after August 6. In both samples the identical structure and composition were observed both by measurements and microchemical tests of the seed coat and peridermium thicknesses. It was apparent in cutting the sections, however, that the seed last collected had a harder coat. By soaking in concentrated sulphuric acid, the carpels of the seed collected August 6 decomposed sooner. The tests showed that the composition of the main hard seed-coat layer in both cases had not progressed beyond the formation of cellulose and that the cells of endosperm and embryo of both samples were filled with proteid. It was not necessary to use the microscope to determine that the endosperm of the early seed did not fill the carpel as well as that of the seed collected later.

Before concluding, it should be emphasized that a higher quality of seed has been produced from year to year by avoiding too high temperatures during extraction, and molding in storage. Investigations with this and the other species, as well as experiments con-

ducted with *Pinus sylvestris* at Eberswalde⁵ and elsewhere, prove that a temperature above 115° F. is undesirable, particularly because of the greatly increased heating capacity of the moister air within the cones. The seed used in these experiments has been extracted promptly under temperatures below 90° F. and preserved from any other possible injury.

SUMMARY

This investigation has indicated that the delayed germination of western white-pine seed is not caused by any inherent physiological characteristic of the seed embryo or endosperm, such as after-ripening or the need for a period of rest, but by the impermeability of the seed coat, and that prompt germination may be obtained from unimpaired fresh seed by any of the following methods:

(1) Reduction of the seed coat by chemical corrosion, preferably by immersion for 45 minutes in concentrated sulphuric acid with thorough rinsing.

(2) Reduction of the seed coat by a mechanical abrasion or pearling process in such a manner that the seed is not cracked or subjected to much pressure.

(3) Soaking the seed in cold water for 24 hours and exposing it to air freezing in winter for at least 40 days.

(4) Burying the seed in moist bark-free sawdust, and maintaining it at a relatively warm temperature for three weeks, followed by immediate sowing.

In the absence of preliminary treatment, good germination may result from late fall sowing of good, newly collected seed, but rainy winter weather with temperatures above freezing may cause the seed to decay.

⁵ HAACK, —. ÜBER DIE KEIMUNG UND BEWERTUNG DES KIEFERSAMENS NACH KEIMPROBEN. Ztschr. Forst u. Jagdw. 38: 441. 1906.

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No. 10

LONGEVITY OF THE TELIOSPORES AND ACCOMPANYING UREDOSPORES OF *CRONARTIUM RIBICOLA* FISCHER IN 1923¹

By PERLEY SPAULDING, *Pathologist*, and ANNIE RATHBUN-GRAVATT, *Junior Pathologist, Office of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

This paper gives the results of investigations of an isolated problem connected with the life history of the white-pine blister rust. It may not be amiss, therefore, to indicate its relation to the general problem. These investigations show that the teliospores, which give rise to the sporidia (which alone can infect pines), may survive outdoor conditions from the time of the formation of the first ones in the season until after winter has set in. Little is yet known of the period during which pines may be infected, but it is presumed that it includes the period of telial formation and possible germination. It is important, then, to know how long and how late in the season teliospores may survive and form sporidia, and the probability of their doing so.

In the late fall and early winter of 1921 the senior writer tested the longevity of the teliospores of *Cronartium ribicola* Fischer (16).² While the results were satisfactory for the period covered (September 21 to December 8), it was felt that such tests should begin earlier in the season of production of the teliospores (17, p. 63) in order to get an idea of the maximum telial longevity under natural conditions. What may happen to the teliospores which are formed earliest in the season has received little direct attention, and this may be of considerable importance. Accordingly, in 1923 the writers made another study with this especially in mind. These tests began August 1, which was the earliest date at which a large enough supply of telial columns could be obtained, and continued until October 31. From August 1 to October 10 the work was carried on at Warrensburg, N. Y., and from October 10 to 31 at Bethel, Vt., under similar conditions. When the experiments were discontinued on October 31 the teliospores in several lots of material were still germinating strongly. The work of 1921 (16) showed conclusively that teliospores of this fungus will readily survive and germinate under the conditions of early winter in Vermont. Judging from the vigor of their germination and the fact mentioned in the preceding sentence, there was no reason to suppose that these persisting teliospores in the present experiments would lose their viability for several weeks after October 31. In view of these conclusions and the pressure of other work which required that these tests be concluded as soon as possible, no attempt was made to continue until the viability of all of the teliospores was exhausted.

¹ Received for publication Mar. 3, 1925; issued January, 1926

² Reference is made by number (italic) to "Literature cited," p. 1015.

THE MATERIAL

CHARACTER OF THE MATERIAL

Twenty-one collections of leaves bearing abundant telial columns were obtained from eight species of *Ribes* (see Table IV for their names)³ in various parts of New Hampshire and northern New York. This material was about as unfavorable for our purpose as could ordinarily be obtained, because in some places there were prolonged local droughts which resulted in sparse telial production and prevented obtaining freshly matured columns (17, p. 63). Pennington (13) in 1920, because of the evenly scattered distribution of the rains during that summer, could get but few viable sporidia of *Cronartium ribicola*, in an area which generally is heavily infected, before the first week of September. In other places it rained frequently enough to partially germinate the telial columns as soon as they were formed.

The amount of pregermination (i. e., germination previous to the collection of the infected leaves) of the telial columns varied greatly. The average pregermination of the different collections of leaves ranged from 0 to 60 per cent. In most cases it was below 15 per cent. On the different leaves of a given collection the pregermination often varied from 0 to 100 per cent of all of the telial columns examined. There was also a similar variation in the average pregermination of the telial columns on the different parts of a single leaf. A telial column which showed one or more germinated teliospores was counted as pregerminated. In such a column there might be left a thousand (19) or more teliospores which were perfectly viable when placed under favorable conditions for germination in the subsequent tests.

In damp-chamber tests made immediately after collection of the material, the average germinability of the telial columns (based upon the column as a unit) varied in the different collections from 7 to 97 per cent. In more than half of the collections it was over 70 per cent. The viability of the teliospores on different leaves and on different parts of the same leaf varied just as did the pregermination for the same units.

While these conditions hindered the writers in getting entirely consistent results, and certainly prevented the obtaining of maximum longevity, they also forestall any criticism for having worked with unduly favorable material.

CONDITIONS DURING STORAGE OF THE MATERIAL

The infected *Ribes* leaves were taken to the field laboratory as soon as feasible after collection. There they were laid singly upon newspapers and allowed to dry in the open air, where they were protected from direct sunshine. As soon as the leaves were wilted, but not crisp, they were placed in mosquito-netting bags and hung out of doors fully exposed to the rain and wind but not to very much sunshine. After rains, when the leaves had begun to dry, they were shaken up so that they would not become matted into a solid mass. An attempt was made to simulate the conditions affecting naturally fallen leaves which had caught among shrubbery in the forest.

³ The nomenclature followed is that given in Coville and Britton (6).

At Warrensburg, N. Y., rain fell in quantities sufficient to wet the foliage on 26 different days, while at Bethel, Vt., this occurred on but 2 days. At the former place there were 7 periods of more than 36 hours when the relative humidity of the air remained above 70 per cent. This condition is shown by the work of the writers to be favorable for the sporidia to retain viability for a long time, and is discussed in their paper on the sporidia of *Cronartium ribicola*.⁴

SAMPLING THE MATERIAL

In order to have representative material for each test, about half of the leaves used in each sample were taken from the middle of the storage bag and the remainder from the outside. The samples were taken after the dew had evaporated. Each sample was placed in a clean, dry Petri dish.

PRECOOLING THE MATERIAL BEFORE TESTING IT

Because it has been generally supposed that precooling per se stimulates the germination of the spores of the Uredineae (7, 8, 9, 10, 16, 17, 21), it was thought best to precool the material before starting germination of the teliospores. In the first two tests this was done by placing the Petri dishes containing the leaf samples in an ordinary refrigerator for about 24 hours. The material for the other tests, with the exceptions noted in Table IV, was placed in the Petri dishes and these then put in a metal container, which was packed in cracked ice and coarse salt for about 24 hours. The temperature thus obtained was certainly lower than that in the refrigerator, although no records were kept. The nightly hard frosts, which began about October 10, were considered to have the desired cooling effect, and the material was not further precooled in the tests run after that date. There was a marked increase in germination of some of the material beginning October 17, the date of the first test run after the frosts began. A suggested explanation is given below.

Table I gives a comparison of germination of precooled and of nonprecooled telial columns. The results are fragmentary because the work was done late in the season, when suitable material was scarce; earlier it was supposed that the effect of precooling was thoroughly established by other workers, and the need for further testing this point was not realized. In the tests recorded in Table I, half of the tested material was precooled by packing the Petri dishes containing it in a mixture of salt and ice for about 24 hours; the other half of the material was kept at room temperature for the same length of time. The two methods of comparing the germination of the two samples of material show that the germination was practically equal in the first two lots of Table I which are designated there as "Fresh telial columns" and "Old telial columns stored indoors." In the lot designated as "Old telial columns stored outdoors," the same methods of comparison show that the uncooled telial columns germinated better; that is, those stored at relatively low temperatures and then subjected to higher temperatures reacted

⁴SPAULDING, P., and GRAVATT, A. RATHBUN. THE INFLUENCE OF PHYSICAL FACTORS UPON THE VIABILITY OF THE SPORIDIA OF *CRONARTIUM RIBICOLA* FISCHER, 1925. [Unpublished manuscript.]

more decidedly than those from a part of the same material in which the low temperatures were followed by still lower ones. So far as these tests go, the writers find that there is no indication that pre-cooling per se of the teliospores stimulates their subsequent germinability. On the contrary, there is some indication that the effect is rather that of decided variation of the temperatures. This suggestion is further supported by the increases in germination which resulted in the tests beginning October 17, apparently as a result of repeated sharp alternation of high daytime and low night temperatures during a period of three weeks with but two days of rain. Eriksson, who seems to have been the first to test the effect of pre-cooling on the spores of *Cronartium ribicola* (9), got a decided reaction as a result of his treatment of the spores, but he does not definitely attribute it to precooling per se or to variation of the temperature. He inclines to the idea that rain just before the spores are collected for cooling has a deciding influence in the reaction obtained.

TABLE I.—Comparison of the germination of precooled and uncooled telial columns of *Cronartium ribicola*

Condition of material at the time of testing	Total number of tests	Number of tests in which precooled telial columns germinated better than uncooled ones	Number of tests in which precooled telial columns germinated poorer than uncooled ones	Number of tests in which neither germinated	Actual germination			
					Precooled telial columns		Uncooled telial columns	
					Number	Per cent	Number	Per cent
Fresh telial columns.....	9	5	4	0	$\frac{525}{1065}$	49	$\frac{406}{930}$	44
Old telial columns stored indoors.....	11	4	5	2	$\frac{95}{1752}$	5	$\frac{106}{1783}$	6
Old telial columns stored outdoors.....	13	0	11	2	$\frac{210}{2148}$	10	$\frac{540}{2163}$	25

* Numerators indicate number of columns which germinated; denominators, the number counted.

The writers' results are merely suggestive. The effects of pre-cooling per se, of decided temperature changes, and of sharp alternations of temperatures on fresh material, on material stored at cold temperatures, and on material stored at warm temperatures should be investigated further.

GERMINATION OF THE TELIOSPORES

OBSERVATIONS ON THE GERMINATION OF THE TELIOSPORES¹

The telial columns and the teliospores are distinct units, and there is no relation between the percentage of telial columns which show germination and the number of teliospores per column which germinate. Throughout the tables in this paper the numerators of the fractions indicate the number of columns which germinated, while the denominators show the total number of columns counted. In

¹ The citations in this chapter are arranged chronologically.

columns 4, 7, etc., of Table IV, and in similar columns of our other tables, X indicates that less than one-third of the visible teliospores in the column germinated; XX indicates that between one-third and two-thirds of the visible teliospores germinated; while XXX indicates that more than two-thirds of them germinated. This method of estimating the number of germinating teliospores is not satisfactory, but no really desirable method of doing this has yet been devised (10, 17).

While the present paper is concerned primarily with the longevity of the teliospores, the writers' observations on their manner of germination, etc., may well be mentioned here. The following phenomena have been noted more or less frequently in these tests. They are not new to the writers, and the references indicate others who have made similar observations. They are chosen especially for work with *Cronartium ribicola*, and in default of this, with some other species of *Cronartium*.

The teliospores germinate normally in situ in the telial column, and with the latter still upon the host leaf (15, 5, 4). The basal spores in a telial column are the youngest, and germination proceeds in each column from the tip downward as the teliospores mature (20, 15, 5). For this reason we may have a telial column at the tip of which are teliospores which have germinated, and still have in it other teliospores which will readily germinate when the necessary favorable conditions are furnished. Each teliospore normally puts forth a germ tube. If the tube immediately reaches the air, it soon divides into a four-celled promycelium (20, 14, 15, 5, 4, 17). If the teliospore is immersed in water, it may fail to germinate at all, or may produce a long, slender tube, which appears to try to reach the air (5, 4). Sometimes this tube forms a four-celled structure at its end, the cells of which soon fall apart, forming what are evidently resting spores (15, 4). If the germ tube from the teliospore should reach the air, it immediately forms a four-celled promycelium. Air seems to be necessary for the formation of normal promycelia. As the germ tube elongates from the teliospore, and especially when the promycelium is formed, the protoplasm of the teliospore enters the tube and fills its outer end (20, 15, 5, 4). The four cells of the promycelium receive this protoplasmic mass, and when they in turn germinate the protoplasm migrates to the ends of their germ tubes. If secondary or tertiary sporidia are formed, the protoplasm continues to migrate to the outermost structure, leaving those behind empty. Normally each promycelial cell germinates, forming a germ tube. This soon forms a small, round conidium called a sporidium (20, 14, 15, 5, 4). The sporidium germinates by putting forth a germ tube; this may quite frequently produce a secondary sporidium nearly as large as the primary one. Sometimes the process is repeated and a tertiary sporidium formed (14, 15, 21, 5, 4, 17). In the experiments described in this paper, secondary sporidia were noted in cultures of teliospores from *Ribes americanum*, *R. glandulosum*, *R. nigrum*, *R. rotundifolium*, *R. triste*, and *R. vulgare*. They were not noted in material from *R. cynosbati* and *R. odoratum*.

In some of their tests the writers noted the effect of massing of the telial columns in decreasing the germination of the teliospores, which is mentioned by Brown (3) for spores of *Botrytis cinerea*.

That is, it was noticed that germination occurred only around the edges of masses of columns. This did not hold in all cases, but was frequent enough to attract attention.

METHODS OF GERMINATION

The original plan was to germinate the teliospores by placing the *Ribes* leaves, bearing the telial columns, upside down in moist chambers until the teliospores had germinated, and then to scrape off the columns for examination, as had been done previously (16). However, a series of 29 parallel tests showed that the "floating" method of germination described below gave decidedly better results than did the moist-chamber method. (See Table II.) In but one instance was germination in the moist chamber better than germination by the floating method. Therefore the floating method was adopted and used for practically the entire series of experiments.

TABLE II.—Comparison of germination of telial columns of *Cronartium ribicola* by the "moist-chamber" and "floating-on-water" methods

Ribes species	Number of collections	Number of tests	Number of days on which tests were made	Number of times better	Germination	
					Number	Per cent
Americanum:						
Water.....	2	4	4	4	204/222	92
Moist air.....	2	4	4	0	235/530	44
Cynosbati:						
Water.....	1	2	2	2	93/142	66
Moist air.....	1	2	2	0	48/455	11
Glandulosum:						
Water.....	2	3	2	3	343/375	92
Moist air.....	2	3	2	0	69/410	17
Nigrum:						
Water.....	4	14	5	13	886/1,458	61
Moist air.....	4	14	5	1	457/3,000	15
Odoratum:						
Water.....	1	2	2	2	145/185	78
Moist air.....	1	2	2	0	29/480	6
Rotundifolium:						
Water.....	1	2	1	2	152/205	74
Moist air.....	1	2	1	0	57/225	25
Vulgare:						
Water.....	1	2	1	2	172/180	96
Moist air.....	1	2	1	0	12/120	10
Total:						
Water.....	12	29	7	28	1,995/2,767	72
Moist air.....	12	29	7	1	907/5,220	17

The floating method of germinating spores was not originated by the writers (14, 1, 2, 9, 3, 11).⁶ It is apparently an outgrowth of the hanging-drop method. Most writers who have used the hanging-drop method do not clearly state whether the spores floated on the surface of the liquid or were immersed in it in their experiments, but a few clearly differentiated between the two (14, 1, 15, 7). Barclay (1, 2) seems to be the first to definitely state that he floated the spores on the surface of the water. H. H. York was the first to use it for spores of *Cronartium ribicola*.

In these experiments a surgeon's cataract knife, with an arrow-head shaped, double-edged blade not more than one-fourth inch long, was

In this paragraph the citations are arranged chronologically.

found best for scraping the columns from the leaves to the surface of water in small glasses containing 10 to 35 c. c. each, according to their size. The telial columns for each glass of water were taken from all of the leaves of a given sample. The leaves should be slightly damp so as to be pliable without breaking, but not wet enough so that the knife edge will scrape water from the leaf tissue, as this will wet the columns and they will sink in the water when dropped on the surface of it. When they sink they germinate little or not at all, as is true of rust spores in general (1, 2, 3, 7, 10, 11, 14). But if dry, they will float readily and for a long time.

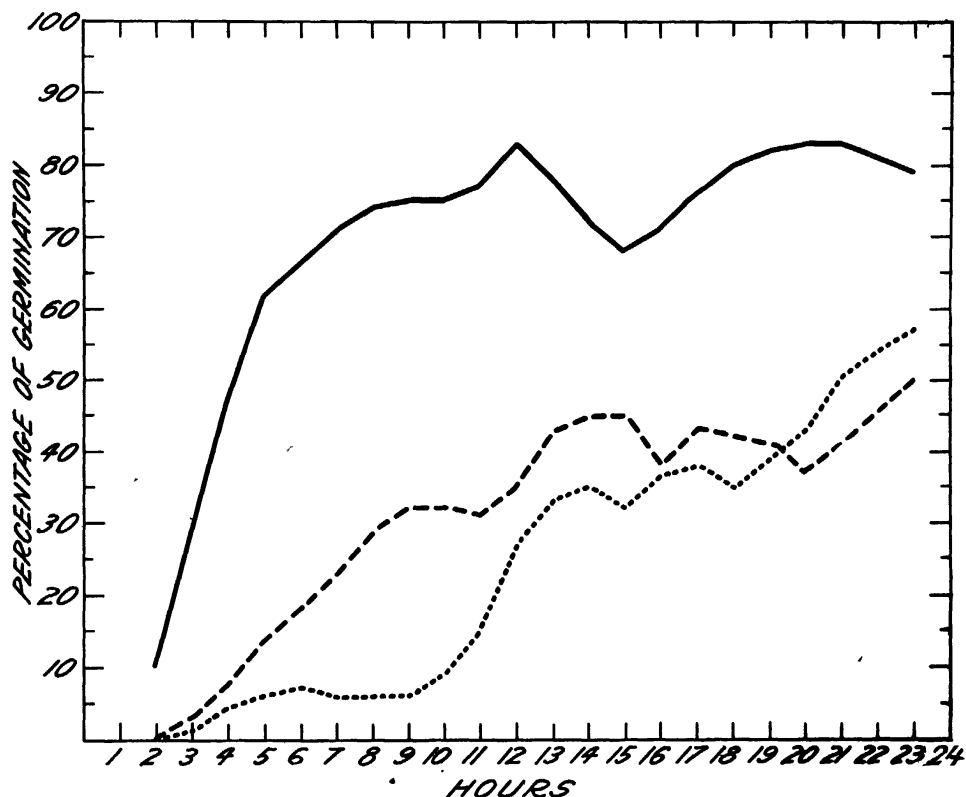


FIG. 1.—Diagram showing time necessary in preliminary tests for the germination of telial columns of *Cronartium ribicola*. The curves are based on 3-hour moving averages, and represent the per cent of the total number of telial columns germinating. The solid line represents the results from telial columns produced on *Ribes nigrum* stored indoors 5 days. The dash line represents those from *R. americanum* stored indoors 15 days. The dotted line represents those from *R. nigrum* stored indoors 25 days.

LENGTH OF THE GERMINATION PERIOD

The teliospores were germinated outdoors, shielded from sun and rain, for a period of 16 to 18 hours. Under the conditions of this work, this length of time was necessary for the maximum amount of germination to take place. This period was unexpectedly long in comparison with 6 hours required in previous years to germinate freshly matured teliospores (22, 17).

Some tests were made to determine the hourly germination of telial columns which had been stored for various lengths of time. Table III and Figure 1 give the results of these tests. The writers' experience shows plainly that the time necessary for germination increases directly with length of time of storage in a dry condition.

TABLE III.—Time necessary for germination of teliospores of *Cronartium ribicola*

Ribes species	Collected		Age (days) when tested	Hours to first germination	Hours to maximum germination of columns
	Place	Date, 1923			
Nigrum.....	Edinburg, N. Y.....	Sept. 30	5	2	12
Americanum.....	Warrensburg, N. Y.....	Sept. 18	15	4	14
Odoratum.....	Darrowsville, N. Y.....	Sept. 12	20	4	18
Nigrum.....	Edinburg, N. Y.....	Sept. 7	25	4	23
Do.....	Wadhams, N. Y.....	Aug. 23	29	5	23

EFFECT OF ALTERNATE WETTING AND DRYING ON THE TELIOSPORES

A survey of the literature shows that considerable work has been done on the effect of alternate wetting and drying upon the teliospores of various genera of the rusts (11). But it is also found that practically all of this work has been done with those teliospores which require a resting period before germinating. Experiments with teliospores which germinate upon maturing, and especially with teliospores of any species of *Cronartium*, seem to be scarce. Since the writers' material was exposed to wetting by dew and especially by rain, and to the subsequent drying due to the weather conditions of the locality, this question became of some importance. The observations made by the writers seem to indicate that teliospores of *Cronartium ribicola* which do not start to germinate perceptibly are not noticeably injured by subsequent drying. When a teliospore once germinates perceptibly, drying seems to kill it. Unless teliospores start to germinate, they survive wetting and drying for an indefinite number of times, as the writers' material was wet many times, as shown above under "conditions during storage of the material." One collection of telial columns of *Ribes nigrum*, stored outdoors, germinated for 70 days while some of the same material kept indoors lived only 49 days, showing that the repeated wetting to which the former was subjected certainly did not shorten the life of the teliospores.

LONGEVITY OF THE TELIOSPORES

Teliospores from eight species of *Ribes* were included in these tests as compared with those from five species of *Ribes* in 1921 (16). Tables IV and V give the results of the weekly germination tests which were made to determine the longevity of the teliospores. The latter also includes the maximum longevity which was attained for each species of *Ribes* in 1921. The amount of germination of telial columns did not decrease regularly with increase in age in several collections of leaves. This was due partly to the variation in the amount of pregermination which had occurred and partly to irregularity in the germination which occurred while the material was kept outdoors exposed to the weather. The character of the *Ribes* leaves bearing the telial columns had something to do with the longevity of the teliospores (18).

TABLE IV.—Longevity of teliospores of *Cronartium ribicola*

[Figure following species indicates collection number]

Date tested	Ribes species, place of collection, and date collected								
	R. nigrum (1), Wevertown, N. Y., Aug. 1, 1923 (from bushes; stored inside)			R. nigrum (2), Wevertown, N. Y., Aug. 1, 1923 (from ground)			R. triste (3), Wilmington Notch, N. Y., Aug. 3, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospor es	Columns		Teliospores
Num-ber	Per cent	Num-ber		Per cent	Num-ber		Per cent		
Aug. 8, 1923	51	47	XXX				28	16	X-XXX, mostly XX.
	108						175		
Aug. 13, 1923	33	33	XX-XXX, mostly XX.	53	53	XXX	8	9	X.
	100			100			90		
Aug. 22, 1923				41	20	X-XX, mostly X.	24	32	XXX.
				204			74		
Aug. 29, 1923				3	2	XX	1	20	XX.
				155			5		
Sept. 5, 1923	36	10	X	9	3	X	16	14	X-XX, mostly XX.
	350			370			115		
Sept. 12, 1923	25	17	X	13	5	X-XX, mostly X.	2	2	X.
	144			267			102		
Sept. 19, 1923	18	7	X-XX, mostly X.	0	0	0			
	248			119					
Sept. 26, 1923	2	1	X	1	1	XX			
	255			125					
Oct. 3, 1923	2	1	X	0	0	0			
	239			130					
Oct. 10, 1923	0	0	0	0	0	0			
	165			135					

Date tested	Ribes species, place of collection, and date collected									
	R. glandulosum (4), Wil- mington Notch, N. Y., Aug. 3, 1923			R. rotundifolium (5), Warrensburg, N. Y., Aug. 4, 1923			R. cynosbati (6), War- rensburg, N. Y., Aug. 6, 1923			
	Germination									
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores	
Num- ber	Per cent	Num- ber		Per cent	Num- ber		Per cent			
Aug. 8, 1923	13 400	3	X	1 255	1	X	33 42	78	XXX.	
Aug. 15, 1923	29 40	72	X-XXX, mostly XX.	10 235	4	X	60 100	60	XX.	
Aug. 22, 1923	16 42	38	XX	5 150	3	XX	9 150	6	XX.	
Aug. 29, 1923	11 175	6	X-XX, mostly X.	0 95	0	0	18 195	9	XX.	
Sept. 5, 1923	39 160	24	X-XX, mostly X.	0 180	0	0	15 240	6	X-XX, mostly X.	
Sept. 12, 1923	5 300	2	X	0 180	0	0	6 175	3+	X.	
Sept. 19, 1923	11 240	4+	X-XXX, mostly XX.	0 129	0	0	1 202	1	XXX.	
Sept. 26, 1923	3 165	2	X				7 155	5	X-XX, mostly XX.	
Oct. 3, 1923	0 113	0	0				1 94	1+	X.	
Oct. 10, 1923	1 170	1	X				3 300	1	X.	
Oct. 17, 1923 ^d	0 20	0	0				1 30	1	XX.	
Oct. 24, 1923 ^d	0 12	0	0							

* Throughout table numerator = number of telial columns which germinated; denominator = number of telial columns which were counted.

† Throughout table XXX=germination of more than two-thirds of the visible teliospores; XX= germination of between one-third and two-thirds of the visible teliospores; X=germination of less than one-third of the visible teliospores.

^a Germination in moist chambers.

^d No precooling.

* Tests at Bethel, Vt.

TABLE IV.—Longevity of teliospores of *Cronartium ribicola*—Continued

Date tested	Ribes species, place of collection, and date collected								
	R. nigrum (7), Wevertown, N. Y., Aug. 6, 1923			R. nigrum (8), Wadhams, N. Y., Aug. 6, 1923			R. odoratum (9), Warningsburg, N. Y., Aug. 7, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
Number	Per cent	Number		Per cent	Number		Per cent		
Aug. 8, 1923	97	97	XXX						
Aug. 15, 1923	100 66	66	XXX	91 100	91	XXX	68 75	91	XXX.
Aug. 22, 1923	100 115	32	Mostly XXX.	94 200	47	XX	47 235	20	XX.
Aug. 29, 1923	360 166	54	XX-XXX, mostly XXX.	132 250	53	X-XXX, mostly XX.	25 145	17	XX.
Sept. 5, 1923	310 115	59	XX-XXX, mostly XXX.	19 210	9	X	1 170	1—	XXX.
Sept. 12, 1923	195 94	25	X-XX, mostly XX.	27 411	7—	XX	2 292	1—	X.
Sept. 19, 1923	375 70	24	X-XX, mostly XX.	23 260	9—	X-XX, mostly X.	3 311	1—	X.
Sept. 26, 1923	291 59	39	X-XXX, mostly XX.	27 191	14	X-XX, mostly XX.	0 200	0	0.
Oct. 3, 1923	150 41	20	XX	6 221	3—	X-XX, mostly X.	0 182	0	0.
Oct. 10, 1923	205 4	2+	X	2 250	1—	X	0 115	0	0.
Oct. 17, 1923 ^{d e}	165 93	33	XX	0 388	0	0	0 77	0	0.
Oct. 24, 1923 ^{d e}	280 39	14	X	0 280	0	0	0 200	0	0.
Oct. 31, 1923 ^{d e}	281 60 280	21	XX						

Date tested	Ribes species, place of collection, and date collected								
	R. nigrum (10), North Conway, N. H., Aug. 11, 1923			R. glandulosum (11), Crawford Notch, N. H., Aug. 11, 1923			R. triste (12), Crawford Notch, N. H., Aug. 11, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
Number	Per cent	Number		Per cent	Number		Per cent		
Aug. 15, 1923	0	0	0	12	14	X-XX, mostly X.	70	70	XXX.
Aug. 22, 1923	100 2	20	X	85 5	14	XX	100 75	44	XXX.
Aug. 29, 1923	10 123	88	XXX	37 68	52	X-XXX, mostly XXX.	170 83	64	X-XXX, mostly XXX.
Sept. 5, 1923	140* 38	18	X-XXX, mostly XX.	130 42	27	X-XXX, mostly XX.	130 41	16	X-XXX, mostly XX
Sept. 12, 1923	212 21	15	XX	155 19	12	X-XXX, mostly X.	155 71	24	X-XX, mostly XX.
Sept. 19, 1923	136 30	34	X-XXX, mostly XX.	158 27	15	X-XX, mostly X.	291 37	19	XX.
Sept. 26, 1923	88 3	3	X	177 7	6	X	195 18	12	X-XXX, mostly XX.
Oct. 3, 1923	93			120			145 1	1	X.
Oct. 10, 1923							136 0	0	0.
Oct. 17, 1923 ^{d e}							225 0	0	0.
Oct. 24, 1923 ^{d e}							55 0	0	0.
							0 168		

^c Germination in moist chambers.^d No precooling.^e Tests at Bethel, Vt.

TABLE IV.—Longevity of teliospores of Cronartium ribicola—Continued

Date tested	Ribes species, place of collection, and date collected								
	R. americanum (13), Warrensburg, N. Y., Aug. 13, 1923			R. glandulosum (14), North Hudson, N. Y., Aug. 15, 1923			R. rotundifolium (15), North Hudson, N. Y., Aug. 15, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
Number	Per cent	Number		Per cent	Number		Per cent		
Aug. 15, 1923	61	91	XX-XXX, mostly XXX.						
Aug. 22, 1923	67 82	58	XXX	99	43	XXX	42	41	XX.
Aug. 29, 1923	141 44	67	XX-XXX, mostly XX.	230 119	60	XX	102 70	42	X-XXX, mostly XX.
Sept. 5, 1923	66 95	76	XX-XXX, mostly XXX.	200 144	61	X-XXX, mostly XXX.	165 58	24	XX.
Sept. 12, 1923	125 68	52	X-XXX, mostly XX.	235 21	12	X-XXX, mostly X.	240 35	14	X-XX, mostly XX.
Sept. 19, 1923	132			174 125	64	X-XXX, mostly XX.	251 22	10-	X-XX, mostly X.
Sept. 26, 1923				195 22	11+	X-XX, mostly X.	228 3	2+	X.
Oct. 3, 1923				195 32	15	X-XX, mostly X.	125 1	1-	XX.
Oct. 10, 1923				215 1	1-	X	187 41	1	X.
Oct. 17, 1923 <i>d e</i>				175 92	43-	X	100 0	0	0.
Oct. 24, 1923 <i>d e</i>				214 8	3	XX	78 0	0	0.
Oct. 31, 1923 <i>d e</i>				259 18 378	5-	X	248		

Date tested	Ribes species, place of collection, and date collected								
	R. vulgare (16), North Hudson, N. Y., Aug. 16, 1923			R. americanum (17), Warrensburg, N. Y., Aug. 20, 1923			R. odoratum (18), West- port, N. Y., Aug. 23, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
Num- ber	Per cent	Num- ber		Per cent	Num- ber		Per cent		
Aug. 22, 1923	34	74	XXX.....	60	71	XX.....	-----	-----	
	46			85					
Aug. 29, 1923	112	86	XXX.....	138	69	XX-XXX, mostly XXX.	124	78	*XX-XXX, mostly XXX.
	130			200			160		
Sept. 5, 1923	26	19	X-XXX, mostly XX.	153	57	X-XXX, mostly high XX.	19	6-	X-XX, mostly X.
	140			270			330		
Sept. 12, 1923	103	52	X-XXX, mostly XX.	75	31	X-XX, mostly XX.	4	1+	X.
	198			245			301		
Sept. 19, 1923	52	49	X-XXX, mostly XX.	97	48	XX-XXX, mostly XXX.	38	16-	X-XXX, mostly XX
	107			202			244		
Sept. 26, 1923	9	10-	X-XX, mostly X.	72	55	XX-XXX, mostly XXX.	38	30	XX-XXX, mostly XXX.
	93			130			125		
Oct. 3, 1923	20	17	X-XX, mostly XX.	11	9	XX.....	11	8+	X-XX, mostly X.
	119			127			132		
Oct. 10, 1923	1	1-	X.....	4	2+	X.....	2	2-	XX.
	110			175			101		
Oct. 17, 1923 ^{d e}	0	0	0.....	13	17	X.....	5	5-	X.
	3			77			111		
Oct. 24, 1923 ^{d e}	4	5	X.....	12	4+	X.....	6	2	X.
	76			285			293		
Oct. 31, 1923 ^{d e}	0	0	0.....	36	21	XX.....	0	0	0.
	168			170			250		

^d No precooling.

^e Tests at Bethel, Vt

GENERAL INVESTIGATIONS.

TABLE IV.—Longevity of teliospores of *Cronartium ribicola*—Continued

Date tested	Ribes species, place of collection, and date collected									
	R. nigrum (19), Wadhams, N. Y., Aug. 23, 1923			R. vulgare, (20), Caldwell, N. Y., Aug. 26, 1923			R. cynosbati (21), West Fort Ann, N. Y., Sept. 6, 1923			
	Germination									
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores	
	Number	Per cent		Number	Per cent		Number	Per cent		
Aug. 29, 1922	315	89	Mostly XXX.	93	93	XXX ^d				
Sept. 5, 1923	355 215	68	X-XXX, mostly XX.	100 77	23	X				
Sept. 12, 1923	315 68	35	X-XXX, mostly XX.	340 61	21	X-XX, mostly X.	7 285	2+	X.	
Sept. 19, 1923	196 78	27	X-XXX, mostly XX.	288 47	21	X-XX, mostly X.	4 214	2-	X.	
Sept. 26, 1923	289 89	59	XXX	219 2	5-	X	19 121	16-	XX.	
Oct. 3, 1923	150 105	66	XX-XXX, mostly XX.	41 1	1-	XX	1 118	1-	X.	
Oct. 10, 1923	160 8	3+	X	105 0	0	0	1 110	1-	X.	
Oct. 17, 1923 ^d	225 75	61	XX	50 0	0	0	0 70	0	0.	
Oct. 24, 1923 ^d	123 87	50	XXX	1			0 230	0	0.	
Oct. 31, 1923 ^d	173 43 400	11-	XX							

^d No precooling.

^e Tests at Bethel, Vt.

TABLE V.—Summary of the longevity of teliospores of *Cronartium ribicola*

Ribes species ^a	Number of days living	Per cent of columns germinating on last day tested	Amount of germination in column on last day tested	Number of days living in 1921 tests (16)	Ribes species ^a	Number of days living	Per cent of columns germinating on last day tested	Amount of germination in column on last day tested	Number of days living in 1921 tests (16)
Americanum (13)	^b 31	52	XX		Nigrum (10)	^b 47	3	X	
Americanum (17)	^b 73	21	XX	^b 59	Nigrum (8)	66	0	0	
Cynosbati (21)	35	0	0		Nigrum (19)	^b 70	11-	XX	
Cynosbati (6)	^b 73	3	XX	59	Nigrum (7)	^b 87	21	XX	^b 79
Glandulosum (11)	^b 47	6-	X		Odoratum (9)	44	0	0	
Glandulosum (4)	69	0	0		Odoratum (18)	63	0	0	^b 59
Glandulosum (14)	^b 78	5-	X		Rotundifolium (5)	19	0	0	
Nigrum (in house) (19)	^b 49	1-	X		Rotundifolium (15)	57	0	0	42
Nigrum (in house) (1)	64	0	0		Friste (3)	41	2	X	
Nigrum (from ground) (2)	57	0	0		Triste (12)	54	0	0	
					Vulgare (20)	39	0	0	
					Vulgare (16)	70	0	0	

^a Figure following species name is number of collection given in Table IV.

^b Living when tests were discontinued.

It should be noted in Table IV that the tests of collections 3, 6, 10, 11, and 13 stopped abruptly before the limit of viability was reached. This was due to the lack of material which was scarce at the time of collection.

The germination tests given in Tables IV and V were made at 7-day intervals. Accordingly a lot of material which is said to have lived 35 days really lived a few days longer, since the teliospores actually died between tests. When there was enough material, a lot which gave no germination in a test was retested twice more to insure accuracy.

When the tests were concluded the teliospores on *Ribes americanum* were germinating rather strongly, although they had weakened perceptibly. Those on *R. glandulosum* were still germinating, but were rather weak and evidently approaching the limit of their viability. As in 1921 (16), some of the teliospores from *R. nigrum* were germinating strongly, and, considering their age, relatively abundantly. In view of the results obtained in 1921, there seems to be no good reason for believing that these strongly germinating teliospores would lose their viability before winter set in.

On the other hand, three lots of teliospores from *Ribes nigrum* had died about the first of October. It appears that the teliospores from *R. rotundifolium* may lose their viability rather quickly. However, those from the latter species, collected August 4, were borne on leaves which had both surfaces completely coated with dew at the time of collection. Partial germination had taken place, and the viability of the remaining teliospores was unusually low, as shown by tests made immediately after collection. The telial columns collected August 3 on leaves of *R. triste* and *R. glandulosum* were probably quite old, because of a local drought which stopped telial production and germination (13).

Teliospores on naturally fallen leaves of *Ribes nigrum*, collected from the ground, lived 57 days when kept out doors. This indicates that infected leaves, even when lying on the ground, may be sources of pine infection for a prolonged period. It is well known that such leaves may be blown for some distance (17). These facts complicate the making of direct field observations upon the distance that sporidia may travel and cause pine infections. But such field observations are valuable in determining how far infection may extend, regardless of the manner, from *Ribes* to pines.

Teliospores on *Ribes nigrum*, collected August 23 and stored indoors, lived 49 days, while part of the same material kept out doors exposed to wind and rain gave 11 per cent germination after 70 days. Another lot of material from *R. nigrum*, collected August 1 and kept indoors, gave germination after 64 days.

Early in January, 1924, teliospores from *Ribes* leaves which had been kept dry in folders were tested for viability by the floating method. The collections were as follows: 2 made in 1915; 3 in 1916; 4 in 1919; 3 in 1920; 2 in 1921; 3 in 1922; 5 in 1923; and 4 in 1924 (fresh material from the greenhouse). In most collections, persisting old promycelia were observed in some abundance, but no new germination of teliospores was observed after 24 and 48 hours, except in the 1924 material, in which the germination was very good within 24 hours.

LONGEVITY OF UREDOSPORES

During the summer of 1923 no material was collected primarily for the purpose of studying the longevity of uredospores. However, in the course of the studies to determine the longevity of teliospores it was found that practically all of the leaves which had produced telial columns had also a generous number of uredospores present. It is often found that leaves apparently bearing only telia also have fresh uredospores borne around the bases of the telia. These probably are the source of the new uredinial sori which often appear on new leaves late in the season. With the above material, in each test in which teliospores were germinated, the germination of the accompanying uredospores was also noted. This means that observations were made only on such uredospores as were floating on the glasses of water together with the telial columns.

Tests of longevity of uredospores of other rusts have been made by a number of workers (12). The results with uredospores of *Cronartium ribicola* range from 7 to 270 days (17) under various conditions. Table VI gives the number of days which the uredospores from the various *Ribes* species remained living after telial columns had formed and after the leaves had been picked. From Table VI it will be seen that no germination of uredospores was accomplished on *Ribes triste*, while on *R. nigrum* the uredospores lived as long as 59 days. On leaves collected from the ground the uredospores lived 50 days, and on material kept in the house they remained viable 36 days; hence the length of time that uredospores can live on leaves after teliospores have been formed varied from 0 to 59 days, in these tests of the writers. The uredospores, as explained under discussion of Table IV, actually lived slightly longer than these figures indicate.

TABLE VI.—Longevity of uredospores of *Cronartium ribicola*

Ribes species *	Date of collection, 1923	Last date alive, 1923	Number of days living	Ribes species *	Date of collection, 1923	Last date alive, 1923	Number of days living
Americanum (13).....	Aug. 13	Sept. 5	24	Nigrum (10).....	Aug. 11	0	0
Americanum (17).....	Aug. 20	Oct. 3	45	Nigrum (19).....	Aug. 23	Oct. 10	49
Cynosbati (6).....	Aug. 6	Aug. 8	3	Odoratum (9).....	Aug. 7	Aug. 29	23
Cynosbati (21).....	Sept. 6	Sept. 9	4	Odoratum (18).....	Aug. 23	Oct. 3	42
Glandulosum (4).....	Aug. 3	Aug. 29	27	Rotundifolium (5).....	Aug. 4	0	0
Glandulosum (11).....	Aug. 11	do.	19	Rotundifolium (15).....	Aug. 15	Aug. 20	6
Glandulosum (14).....	Aug. 15	Aug. 20	6	Triste (3).....	Aug. 3	0	0
Nigrum (1).....	Aug. 1	Sept. 5	36	Triste (12).....	Aug. 11	0	0
Nigrum (2).....	do.	Sept. 19	50	Vulgare (16).....	Aug. 16	Aug. 20	5
Nigrum (7).....	Aug. 6	Oct. 3	59	Vulgare (20).....	Aug. 26	Sept. 12	18
Nigrum (8).....	do.	Aug. 15	10				

* Figure following species name is the number of collection given in Table IV.

The viability of uredospores in old material was tested at the same time (January, 1924) and from the same material as here reported for teliospores. Examination of each of several collections showed a very few uredospores with persisting old germ tubes. Spores which had been floated on water had a few similar persisting old tubes but no new ones. The fresh uredospores germinated abundantly. M. W. Taylor found similar persisting germ tubes among old aeciospores of this fungus. In making germination tests of old spores, it is necessary to examine first for these persisting old germ tubes.

SUMMARY

During the summer of 1923 telia from eight *Ribes* hosts were tested for longevity. Under outdoor conditions their longevity varied from 19 days for one collection of *Ribes rotundifolium* to 87 days for *R. nigrum*, which still germinated well at the end of the experiments.

The "floating" method of germination was much superior to the damp-chamber method.

Precooling per se did not seem to stimulate germination of teliospores markedly. Tests are needed to prove whether decided alternations in temperatures are not the stimulating factors.

The period of germination of teliospores increases with their age, whether kept dry indoors or outdoors exposed to the weather.

Uredospores accompanying the teliospores remained viable for a maximum period of 59 days under the conditions of these experiments.

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THE CRITICAL TEMPERATURE FOR INFECTION OF THE POTATO SEED PIECE BY *FUSARIUM OXYSPORUM*¹

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INTRODUCTION

The infection of the potato (*Solanum tuberosum*) seed piece by *Fusarium oxysporum* Schlecht. commonly results in much disease and consequent economic loss in the western part of the United States. Of the factors influencing the development of this disease one of the most important is temperature, for it is an element of the environment to which both host and parasite respond. The effects of other elements are altered by its changes. The critical temperature of infection—that is, the point below which parasitism fails to occur, and above which damage to it develops—is the point of departure for studies of wilting, rotting, and other manifestations of infection.

REVIEW OF LITERATURE

In 1919 MacMillan (8)² pointed out the fact that temperature is vital so far as infection of the potato seed piece by *Fusarium oxysporum* is concerned. Other *Fusaria* and different host plants have received the attention of a number of workers. Gilman (3) determined 17° C. to be the critical temperature of infection of cabbage by *F. conglutinans*. Tisdale (9) found flax to be infected by *F. lini* at 15° or higher. Clayton (1) determined 22° as approximately the critical temperature for infection of the tomato by *F. lycopersici*. Johnson and Hartman (6) reported that the root-rot disease of tobacco less marked below 15°, and that a number of other soil factors influence the development of it. Goss (5) found that the wilt of potatoes caused by *F. oxysporum* did not develop below 18°. In the determination of these temperatures one type of apparatus was used by all, the so-called Wisconsin temperature tank, described by Jones (7).

Under practical conditions no constant temperature of the soil is to be expected. Dickson (2) states that the mean of a fluctuating soil temperature develops the same condition as a constant temperature equal to the mean. But for the exact determination of a critical temperature point as constant a temperature as possible is desired. Goss (4) in a controlled temperature study of *Fusaria* rots maintained the temperatures with a variation of only 2° C. Other workers have allowed variations of these small amounts, inevitable from the nature and duration of the experiment.

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² Reference is made by number (*italic*) to "Literature cited," p. 921.

APPARATUS AND METHODS

The apparatus used for maintaining the temperatures of the experiments herein reported was an adaptation of the Wisconsin tank method. Essentially, it consisted of a series of water baths for small soil cans. A large wooden bin about 4 by 20 feet was built in the greenhouse, and 12 round, galvanized tanks, each 16 inches in diameter and 26 inches deep, were so arranged in the bin as to allow a maximum amount of space around each tank. The bottom of the bin and the spaces between the sides of it and the tanks were well packed with sawdust and straw as a means of partial insulation. A $\frac{1}{2}$ -inch brass tube was soldered into the side of each tank at the bottom, and a rubber tube attached which reached to the outside of the bin, where it was closed with a spring clamp. The bin and tanks were covered with boards closely fitted. Over each tank three holes 5 inches in diameter were cut, the centers being equally spaced on a circle the radius of which was 3.5 inches, and the center of which was approximately the center of the tank. At the center and between the three large holes a small hole was made to receive a thermometer. Thirty-six galvanized-iron cans, each 5 by 12 inches, were prepared with a wire lip at the top, so that they would not fall through the hole in the cover above the tank.

The method of preparation of the soil and seed potatoes for use in these cans was simple. A uniform quantity of a suitable potato soil was prepared by much working and shoveling, and brought to a uniform moisture content of approximately 18 per cent. The soil and cans were sterilized by steaming. The soil was cooled and mixed again. Potatoes of the Early Ohio variety were used. They were of uniform size, each weighing about 4 ounces. The tubers were immersed in a 1 to 1,000 solution of mercuric chloride for one hour, then rinsed with sterile water. Following this, they were cut once lengthwise with a sterile knife, inoculated, and planted immediately. One can of the three for each tank was used as a control. The surface of the control seed piece was treated with 2 cubic centimeters of sterile distilled water. The seed pieces for infection were inoculated with 2 cubic centimeters of a heavy spore suspension of *Fusarium oxysporum* in sterile distilled water. Six seed pieces were planted in each can in two layers, three in each layer. The top layer came within 4 inches of the soil surface, and was about 5 inches below the surface of the water in the tank. The cans were brought to uniform weight by the addition of soil. They were then placed in the holes in the cover of the tanks. The tanks had been previously filled to the top with water and adjusted to the desired temperature.

No automatic device was used to regulate or control the temperatures. The tanks were under observation continuously for the duration of the experiment. The thermometers used were carefully calibrated, and corrections compensated for in the readings. The temperature of the water was raised by introducing an electric immersion heater, and lowered by the addition of cold water, with the removal of an equal volume at the bottom. Before and after each change the water was thoroughly agitated with a 4-inch propeller on a long shaft turned by a hand drill. In order to add water and insert the propeller a can had to be lifted. They were lifted in rotation. Thermometers in them detected no change of tempera-

ture in this process, since the time involved was usually less than a minute. The 12 tanks were paired, and six different temperatures maintained. Comparisons between the temperature of the tank and the temperature of the soil in the cans at the depth at which the potatoes were showed them to be the same.

THE PRELIMINARY EXPERIMENT

The temperatures for the preliminary experiment were 14°, 16°, 18°, 20°, 22°, and 24° C. Readings were recorded every 2 hours, although corrections were frequently made oftener. The first experiment ran from 6 p. m. February 16, 1922, to 6 p. m. February 26, 1922, a period of 240 hours. An effort was made to maintain the greenhouse temperature at about 18° C., but it rose during the day and frequently fell during the night. Even with care there were deviations from the desired temperatures, but rarely more than two or three tenths of a degree. The tank at the high-temperature end of the bin came too near the heating pipes, and the temperature was kept down with difficulty.

The average deviation for the period of the experiment is given in Table I.

TABLE I.—*The temperature which it was desired to maintain in each tank in the preliminary experiment, together with the average deviation for the experimental period, expressed in degrees centigrade*

Desired temperature.....	14.0	16.0	18.0	20.0	22.0	24.0
Average deviation:						
First tank.....	+0.049	+0.013	−0.002	−0.003	−0.020	−0.010
Second tank.....	+0.005	−0.049	+0.002	+0.052	+0.027	+0.142

At the termination of the experiment the cans were opened and the seed pieces examined for evidence of infection. The seed pieces in the control cans were clean, the cut surface bright. To all appearances they were about as when planted. The seed pieces inoculated with *Fusarium oxysporum* showed varying degrees of infection, and all of them showed evidence of contact with the fungus. At 24° C. all were well infected, the decay varying in depth from 2 millimeters to 1.5 centimeters, the epidermis was shrunken, and the flesh discolored. At 22° all were well infected, though the infection was not as far advanced as at 24°. At 20° and 18° all pieces were infected in much the same manner as at 22°, but with less penetration into the flesh of the seed piece. At 16° infection was more moderate, all pieces were infected, but decay penetrated only to a depth of about 2 millimeters. At 14° infection was not apparent. The inoculated surface was dark, the tissue was slightly browned just under the surface, and there was no shrinking at the edges. Upon reisolating the fungus it was secured in pure culture from all of the seed pieces maintained at 16° or higher, and from two seed pieces held at 14°.

FINAL DETERMINATION OF CRITICAL TEMPERATURE FOR INFECTION

In order to more carefully determine the temperature at which infection occurs the experiment was repeated, a restricted range of temperatures being used. The cans were sterilized, the soil was pre-

pared as before, potato seed pieces from the same lot of potatoes were prepared in the manner described above, and the thermometers were recalibrated. The tanks were adjusted by pairs to temperatures of 13°, 13.5°, 14°, 14.5°, 15°, and 15.5° C. The duration of the experiment was 300 hours, beginning at 6 p. m. March 9, 1922, and ending at 6 a. m. March 22, 1922. Readings and adjustments were made every hour. The average deviations from the desired temperatures are given in Table II.

TABLE II.—*The temperature which it was desired to maintain in each tank in the final experiment, together with the average deviation for the experimental period, expressed in degrees centigrade*

Desired temperature.....	13.0	13.5	14.0	14.5	15.0	15.5
Average deviation:						
First tank.....	+0.0133	+0.008	−0.0003	−0.0073	−0.0053	−0.0013
Second tank.....	+0.004	+0.0003	−0.0013	−0.0073	+0.0046	+0.0033

At the termination of the experiment the cans were lifted, and the seed pieces removed and examined for evidence of infection. All of the controls appeared distinctly different from the inoculated seed pieces held at the same temperature. The cut surfaces were clean and bright as if almost freshly cut. The inoculated pieces held at 13° were more heavily calloused than the controls, and appeared a trifle darker, but there was no evidence of infection in any case. The buds of the seed pieces had germinated normally. Of the seed pieces inoculated with *Fusarium oxysporum*, none held below a temperature of 14.5° C. appeared to be infected. They seemed to be a little more heavily calloused, and very slightly shrunken about the edges. At 14.5° and above the seed pieces were more or less shrunken at the edges, and the cut surfaces had developed a mottled appearance, frequently characteristic in this infection. The reisolation of the causal fungus from the tissue below the surface was depended upon to show whether or not infection had occurred. Mere examination was insufficient. The tubers were broken open toward the cut surface, and fragments of tissue below the surface, but not including it, were transferred to poured plates of potato agar. Where a fusarium developed it was transferred to suitable tube media and identified. From the seed pieces held at 13° and 13.5° no fungus developed. Of those held at 14°, six pieces, or 25 per cent, yielded *F. oxysporum* in pure culture. At 14.5°, 15°, and 15.5° all seed pieces yielded *F. oxysporum* in pure culture.

The critical temperature for infection of the potato seed piece was found to be approximately 14° C. under conditions such as prevailed during the course of these experiments.

DISCUSSION

The conditions under which the experiments were performed were approximately normal, so far as the soil was concerned. Because infection occurred at 14° C. it must not be assumed that wilt or death of the plant would have taken place had the experiment lasted long enough to have produced plants. Vegetative activity offers resistance to the advance of seed-piece rot. Other factors, such as vigor of seed and aeration, commonly prevent or retard seed-piece

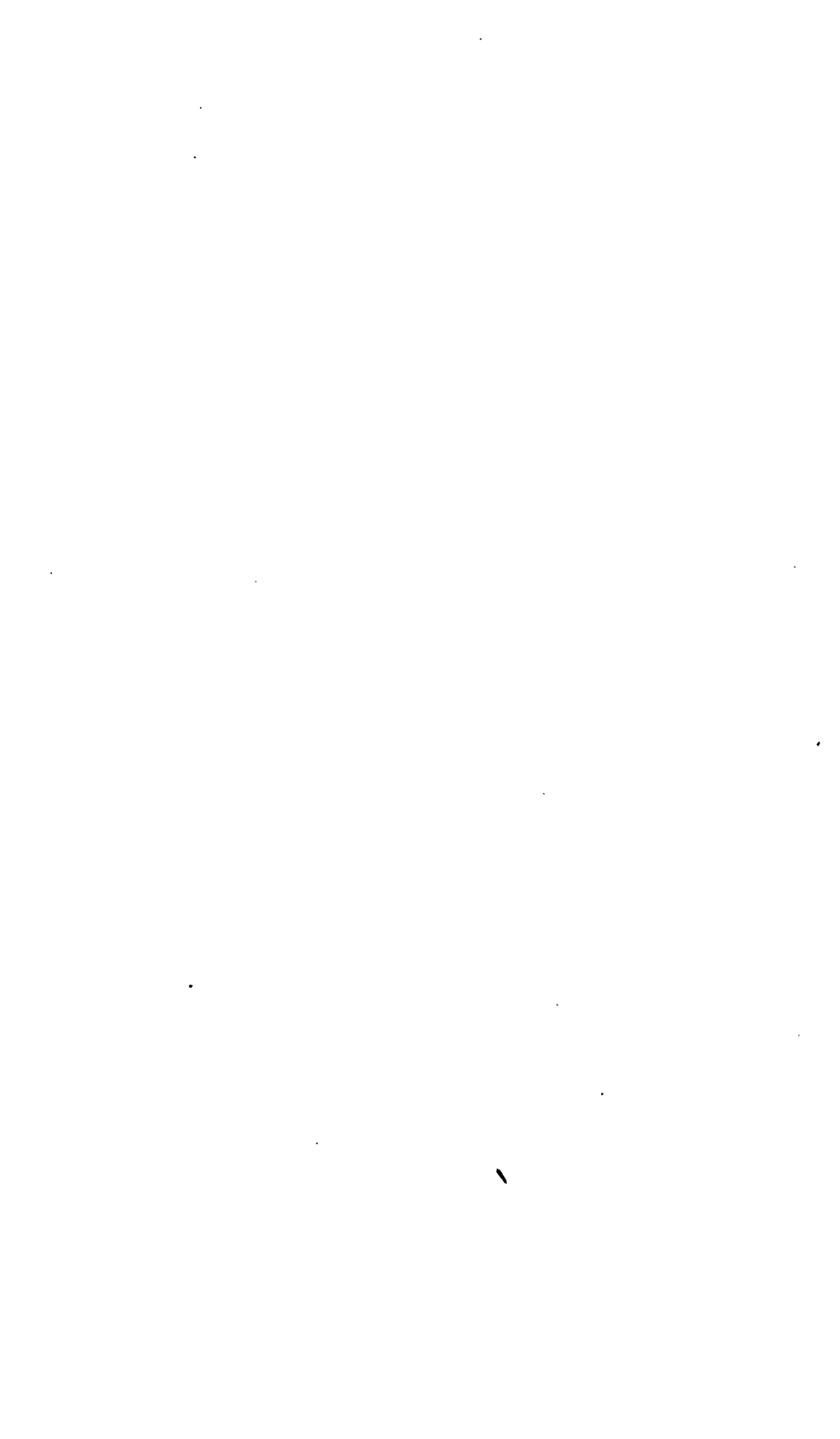
decay, even when the soil temperature is well suited to it; and the fungus is present. As has been pointed out before (8), infection appears to be universal in certain sections of the West, and there is no reason for withdrawing this statement. Decay does not necessarily follow infection at once, and may not over an extended period, but when the proper conditions arise epidemics develop. A low initial infection temperature assists the disease to appear in epidemic form.

CONCLUSION

Under approximately normal conditions and at constant temperatures, seed pieces from potatoes of only moderate resistance are infected by *Fusarium oxysporum* at approximately 14° C., but not at lower temperatures.

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SOME POSSIBLE ERRORS IN THE USE OF CURVES¹

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Although the analysis of data by means of graphs and curves has been one of the most common procedures in forestry, little investigation apparently has been made of the limitations of the method or of the seriousness of possible errors which may result from ignorance or disregard thereof. The basic idea of plotting measurements on coordinate paper and of fitting curves thereto is derived from analytical geometry, and there has been a tendency to assume that any of the processes of the algebraic basis for this branch of mathematical science are equally permissible in the case of empirical curves. Pure mathematics, however, assumes exact equations between the variables under consideration, and these variables are therefore perfectly correlated. Empirical data are never perfectly correlated and are usually (at least in the case of forestry) very loosely correlated. As the degree of correlation becomes less, it can be shown that certain mathematical processes become increasingly inapplicable until in many instances gross errors are introduced by their use.

Probably the best way to make this possibility of error clear is to take a single data series and subject it to apparently plausible transformations, comparing the results in each case with those obtained by a more direct handling. The material selected is taken from measurements of a series of 524 second-growth long-leaf pine trees and, for simplicity, is limited to the three measurements: Diameter (breast high), height, and volume in cubic feet. This is a case in which the variables are comparatively closely correlated and in which, therefore, no abnormally serious discrepancies should occur. Few forestry problems afford better material, and if any important errors are discovered in this instance, difficulties at least as great may be expected wherever parallel processes are used.

Let us consider first the possibility of interchanging the independent and the dependent variables. Texts in forest mensuration have warned against this, but without sufficiently emphasizing the kind and degree of error involved therein. At first glance, it seems quite obvious that if a table or curve showing average heights of trees of given diameters is available, then the average diameters of trees of given heights should be obtainable therefrom. To do so would be analogous to transforming an algebraic equation such as $y = ax + b$ into $x = \frac{y-b}{a}$, and it is probably because of the correctness of such an algebraic transformation that the temptation to perform a similar process with empirical curves is so strong. But let us see the result.

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In Figures 1 and 2 the data series just described are plotted, first with diameter at breast height and then with height as the independent variable. In Figure 1 each cross represents the average

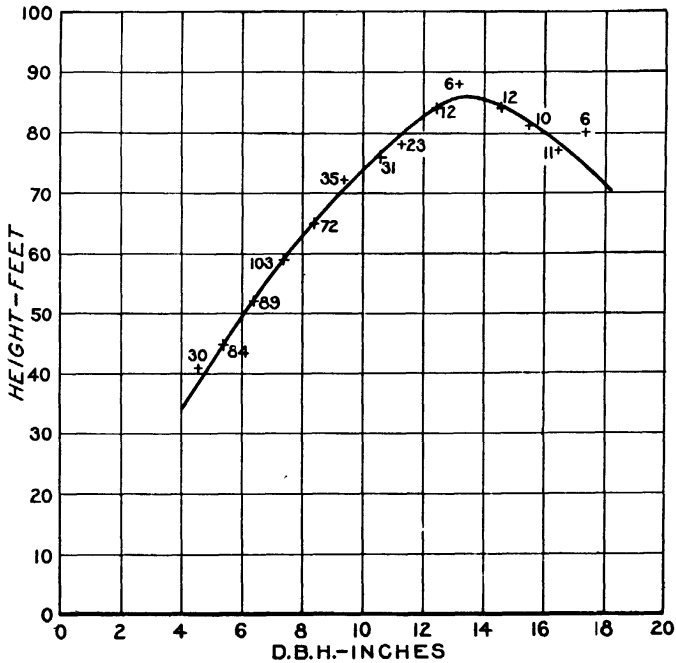


FIG. 1.—Curve of height over diameter breast high

height of all the trees in a single 1-inch diameter class, the adjacent figure indicating the number of trees involved in each average. In Figure 2, similarly, each cross represents the average diameter at

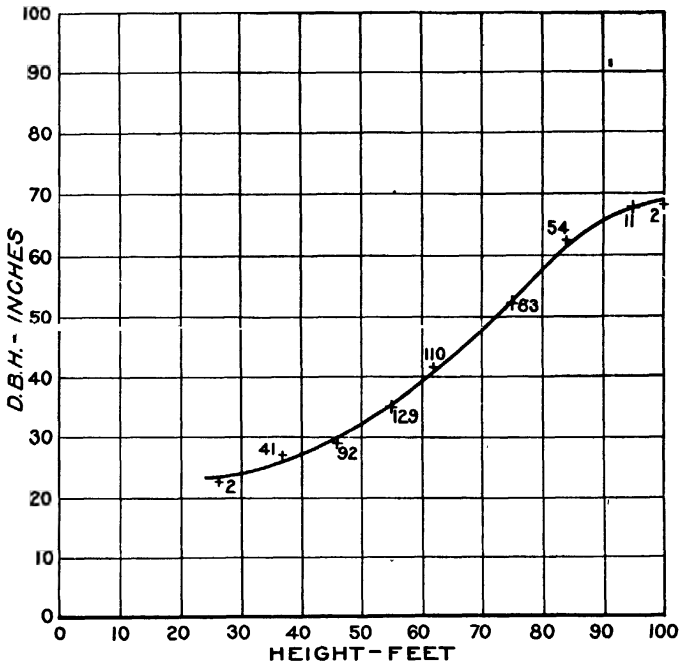


FIG. 2.—Curve of diameter breast high over height

breast height for all the trees in a single 10-foot height class. Each curve is quite strongly defined. Some surprise may be occasioned by the fact that the first of these curves falls after passing 13 inches

in diameter, but this is readily explainable on the ground that the trees of largest diameter were undoubtedly those which were standing isolated from their neighbors, and which were shorter than those growing in denser stands. It should be noted, also, that curve 2 does not necessarily originate from the point 4.5—0, because it is neither a growth curve nor (what is very similar) a relationship curve for a strictly selection stand.

If the two curves are carefully compared it will at once be seen that the one is not merely a reversal of the other. The extent of the difference is best brought out by Figure 3. In this the curve of Figure 1 is replotted, while that of Figure 2 (the broken line) is turned on its side so that its axes coincide with those of curve 1. Through the central part of the curves the errors are relatively small, but the upper end is startlingly different, both in position, trend, and range

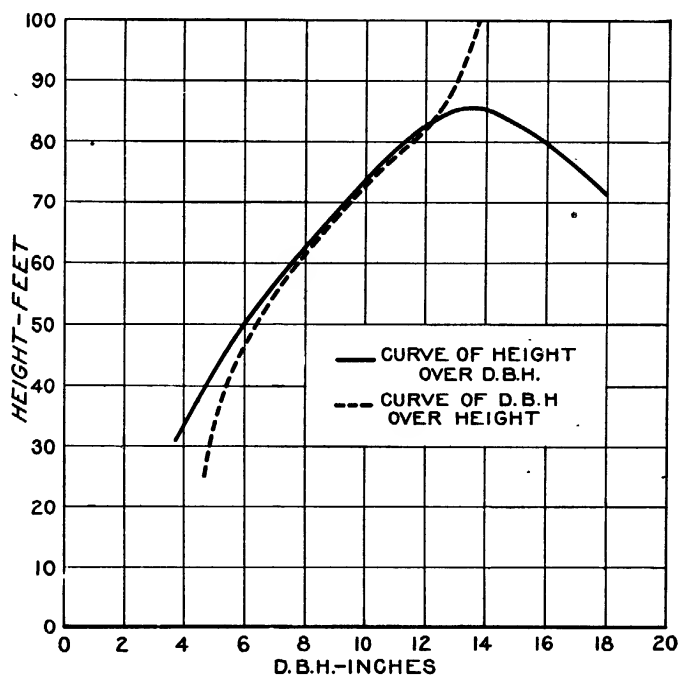


FIG. 3.—Comparison of curves of height over diameter, and of diameter over height, for same data

of values. It is clear that to reverse either curve and use it as a substitute for the other will result in errors which may be very serious. The approximate coincidence of the two curves for the central half of their range is an indication of quite high correlation, but in spite of this the distortion at the upper end is so great that the most interesting fact brought out by the upper part of curve 1 is completely obliterated.

A second type of transformation which seems equally plausible (and on which the texts are silent, if not actually misleading) suggests itself in a case such as the following. Let us assume we have at hand a curve of height over diameter at breast height (such as fig. 1) and one of volume over height (such as fig. 4). Let us also assume that we wish a curve showing the relation between volume and diameter. Apparently we can obtain this curve directly from the two already drawn without having to turn once more to the original data, a process which would involve a considerable amount of labor in

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regrouping and averaging. We have once more an apparent precedent in algebra, for if we have equations between x and z and y and z such as $z = ax + b$ and $y = cz + d$, we may with perfect propriety substitute the value of z from the first equation in the second and obtain, as the equation between x and y ,

$$y = c(ax + b) + d = acx + bc + d$$

But again, let us examine the actual result with our empirical data.

The curve in Figure 5 is thus worked out. For example, from Figure 1 we find that the average height of 4-inch trees is 34 feet. From Figure 4 we find that the average volume of 34-foot trees is 1.6 cubic feet. We may therefore plot 1.6 cubic feet as the average volume of 4-inch trees. In a similar way we may find values for volumes corresponding to as many successive values for diameter as

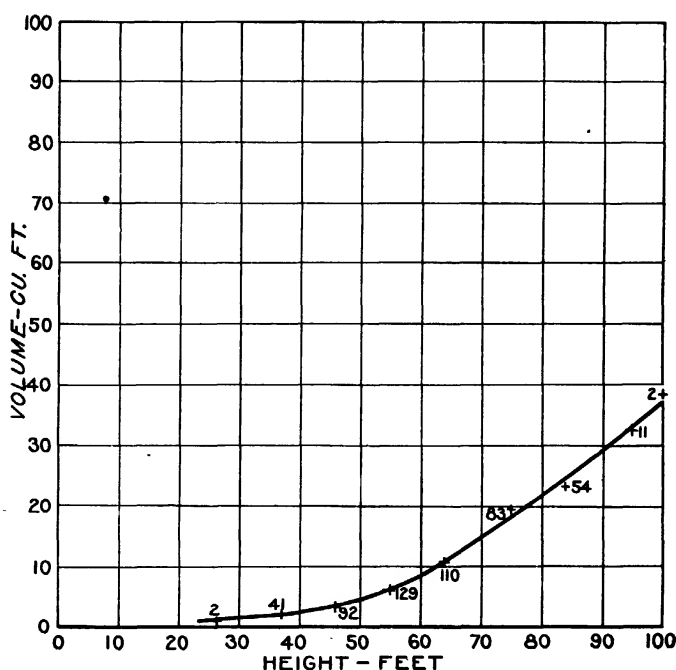


FIG. 4.—Curve of volume over height, for same data

we please. Since the values are derived from two curves they will themselves inevitably fall on a smooth curve.

The best test of the accuracy of this curve is to see how well it fits the data when the latter are handled in the straightforward manner. The same material has therefore been sorted into 1-inch diameter classes and the average value for each class obtained. The resulting values are plotted as small crosses in Figure 5. It is clear that the derived curve is entirely inadequate. Even at the lower end the percentage differences between the plots and the curve are very considerable (over 30 per cent), while at the upper end the discrepancies in both position and trend are gross.

Nor is this accidental. With three variables under consideration six curves are possible (height over diameter, diameter over height, height over volume, volume over height, volume over diameter, diameter over volume). All six of these have been drawn both directly from the data and by the indirect method just described.

In every instance large discrepancies were found, which are so similar in character to those illustrated in Figure 5 that to present them here would introduce no new element.

In both the instances which have been described the way in which the erroneous results are obtained can best be understood by actually working out an example, and carefully noting how the different individual values combine with other values and exert their influence on different parts of the curve. In the last instance, for example, it will readily be seen by inspecting the curves that for any given diameter the particular trees which determine the average height (curve 1) are for the most part entirely different from those which determine the corresponding average volume. The possibility that diversity in manner of grouping is the prime cause of the difficulties encountered suggests an alternative procedure.

Suppose measurements are sorted by height class, and that the average diameter at breast height and average volume for each class are determined. By plotting these average volumes over these average diameters the curve which has been sought can apparently be

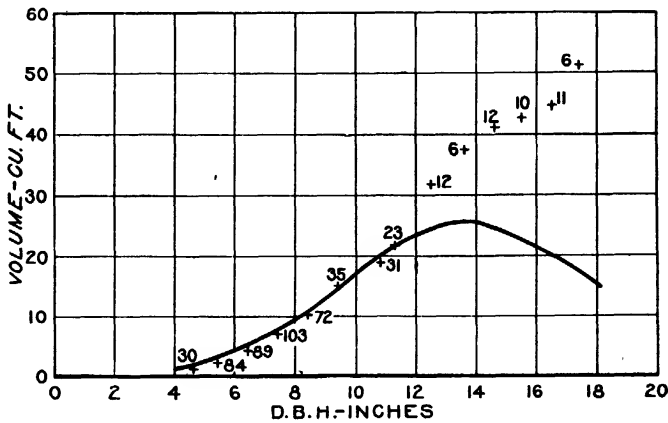


FIG. 5.—Curve of volume over diameter, obtained indirectly from curves of Figures 4 and 1, compared with actual averages

obtained in still a third way. Furthermore, it is immaterial whether actual averages be used or whether advantage be taken of the fact that curves have already been drawn for each of these variables over height by using corresponding curve values. The algebraic analogy would be almost identical with that just described.

For example, we may find from Figure 4 that the average volume of 30-foot trees is 1.3 cubic feet, and from Figure 2 that the average diameter at breast height for 30-foot trees is 4.8 inches. We may then plot 1.3 over 4.8. Repeating the process for other height classes, we produce the volume-diameter curve illustrated in Figure 6. When the actual average values are plotted, however, as has been done in the figure, this curve in turn is seen to be a very poor expression of the data. Particularly noticeable is the fact that the curve covers only about two-thirds of the range of the points and gives no values at all for the higher diameters. In the present instance the result of this last process is perhaps less erroneous than that previously illustrated (fig. 5); but when similar procedures were tried with the other five possible curves, it was found that this superiority was accidental, and that in general there was little or no choice.

It appears, then, that a curve based on empirical data can neither be used backwards nor combined with other curves, without serious liability of gross errors. It also appears that material should not be sorted for averaging in any way except on the basis of the independent variable. The seriousness of the errors depend largely on the degree of correlation between the variables. Yet in the literature of forestry may be found innumerable examples in which analogous processes are involved. The first error is more generally recognized and more generally avoided, although tree taper curves, for example, are still quite generally advocated for obtaining average heights to given

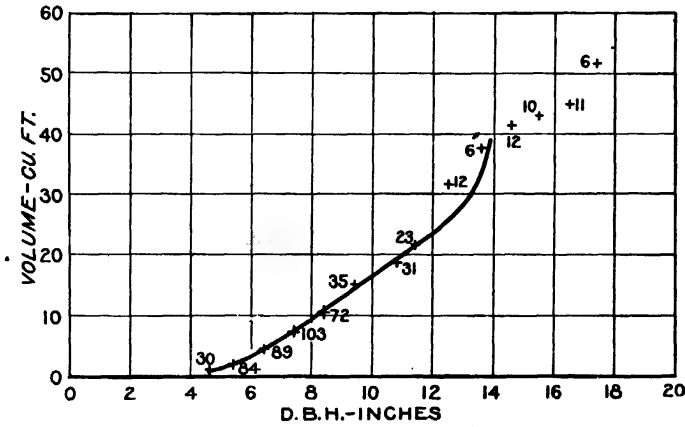


FIG. 6.—Curve of volume over diameter, obtained indirectly from curves of Figures 4 and 2, compared with actual averages

diameter limits, whereas they give accurately only average diameters at given heights. The second error is probably more common than the third. It is exemplified in the study of uneven-aged stands by treating diameter classes as if they were age classes, when once the relation between age and diameter is worked out. In this case the correlation is obviously quite low, and one can but surmise how inaccurate the results thus obtained may be. A determination of the coefficient or index of correlation in such cases may suggest how great the dangers in the indirect treatment are, but reliability can only be obtained by the simple direct method, laborious though it may prove to be.

A PRELIMINARY STUDY OF THE GROWTH OF NOBLE FIR¹

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INTRODUCTION

Noble fir (*Abies nobilis*) is one of the main components of the forest in the upper-slope type on the west slope of the Cascade Range in Oregon and Washington. It rarely occurs in pure stands. In the lower part of its range, it is usually found in mixture with Douglas fir (*Pseudotsuga taxifolia*), western hemlock (*Tsuga heterophylla*), and amabilis fir (*Abies amabilis*). In its upper limits it is associated with western white pine (*Pinus monticola*), amabilis fir, and yellow cypress (*Chamaecyparis nootkatensis*) in Washington and northern Oregon, and with white fir (*Abies concolor*), white pine, and hemlock in southern Oregon. It also occurs in the Olympic Mountains of Washington, and is found as a rare tree in the Coast Ranges of Oregon.

The altitudinal limits of the noble fir lie between 3,000 feet and 5,000 feet, occurring at this higher point only in the southern part of its range. In northern Washington its upper limit is between 4,000 and 4,500 feet. Below 3,000 feet it occurs only as a rare, scattered individual in mixture with the Douglas fir.

The data used in the present study were obtained in connection with a general study of western hemlock upon the logging operation of the Bridal Veil Lumber Co. near Palmer, Oreg., on the west slope of Larch Mountain (T. 1 N., R. 6 E., W. M.)

The area involved presented a west to southwest aspect on a gentle to moderate slope, at an elevation of from 3,000 feet to 3,500 feet. The topography is relatively smooth, and the drainage is very good, the area being cut into gentle contours by a number of small streams.

The soil consists of a clayey to a sandy loam, 6 to 18 inches deep, with a moderate depth of humus and decaying vegetable matter on the ground. The subsoil is composed of rock and gravel, none of the rock outcropping to the surface.

The climatic conditions are such as to be quite favorable to tree growth. The temperature does not vary greatly during the different seasons. The summers are fairly cool, with an abundance of fog and mist; while the winters, although having an excessive snowfall, are not subject to extremely low temperatures. The precipitation ranges from 80 to 90 inches per annum. For the best development of noble fir, a comparatively humid climate and a fresh, deep, porous soil are required. Although this locality is a quality III site of the lower slope type for Douglas fir, it is the best quality upon which noble fir grows, as this tree does not form any stands at lower elevations.

The stand represents a mature forest composed of noble fir, Douglas fir, and western hemlock, with a scattering of amabilis fir. This

¹ Received for publication Dec. 23, 1924; issued January, 1926.

last-named species becomes more abundant with an increase in elevation, taking the place of hemlock and Douglas fir, these species dropping out. Noble fir and Douglas fir form the dominant crown classes, hemlock occurring mainly as an understory. In point of numbers, hemlock forms about 60 per cent of the stand, noble fir and Douglas fir about 20 per cent each; according to volume, however, noble fir forms 46 per cent, Douglas fir 24 per cent, and hemlock about 30 per cent. The crown canopy varies from 0.8 to 0.9 in point of density for the upper story, which, coupled with the canopy of the suppressed hemlock, casts a rather dense shade. This is probably the main reason for the almost total lack of underbrush, except where the crown has been opened owing to windfall or death of the dominant trees.

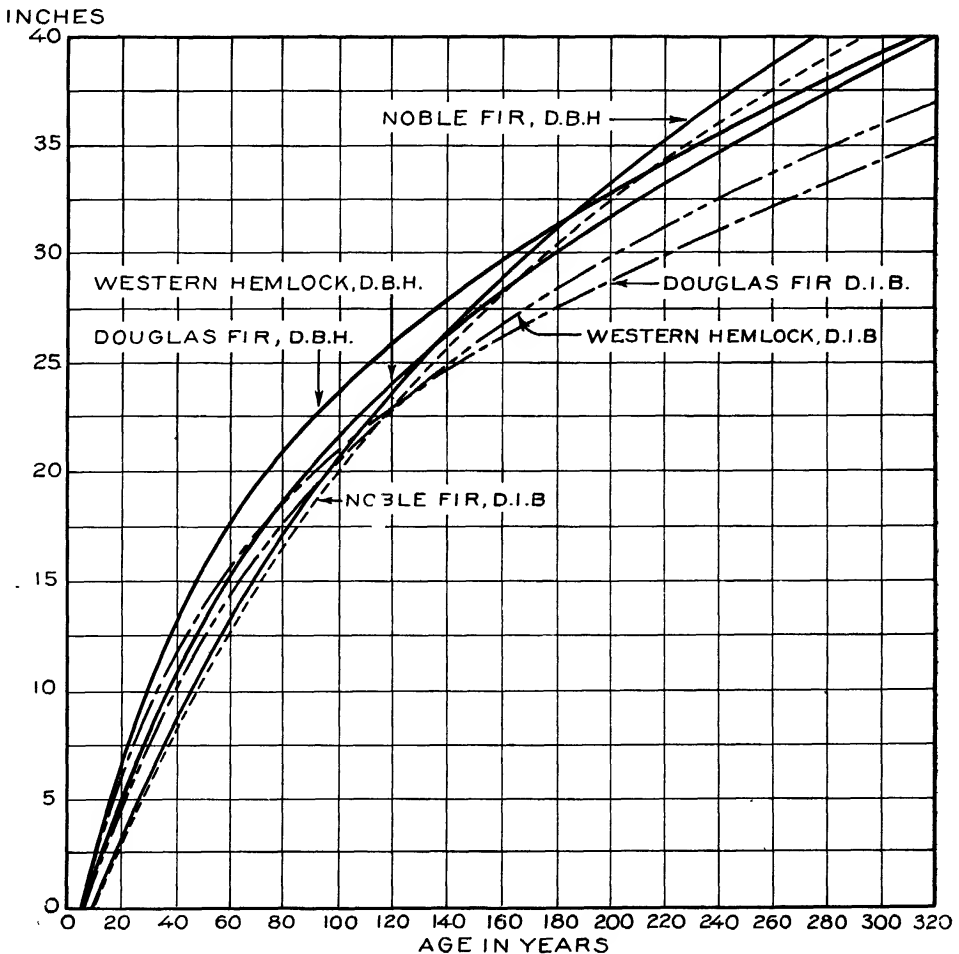


FIG 1.—Diameter-growth curves for noble fir, Douglas fir, and western hemlock

THE TREE AND ITS MANAGEMENT

Noble fir in this locality forms a tall, straight, cylindrical bole with a comparatively small and open crown. The trunk prunes itself readily of side branches, indicating the rather intolerant nature of the tree; the length of the crown being only about one-third to one-fourth of the total height. Trees were found up to 6 feet in diameter at breast height, attaining a maximum height of about 215 feet. The average tree, however, has a diameter of a little more than 4 feet, and an average height of close to 200 feet.

Noble fir is less tolerant than either western hemlock or amabilis fir, its light requirements being about the same as those of Douglas fir. Seedlings are seldom found in the shade of older stands, the undergrowth usually consisting of stunted hemlock and amabilis fir. Noble fir seed will germinate both upon bare mineral soil and on duff, preferably the latter. It requires rather moist soil conditions for best results. Reproduction takes place only in openings, such as are caused by fire or lumbering.

Noble fir is probably best adapted to a mixed type of forest where it is able to maintain a dominant position in the stand, such as when in mixture with Douglas fir and hemlock, or amabilis fir and hemlock. Since it does not reproduce itself under shade, clear cutting in groups or strips is apparently necessary to obtain satisfactory natural reproduction. In the present mature stands, in order to accomplish natural restocking, cutting should take place in the spring following a good seed year. This will insure a crop of seed on the ground which should find favorable conditions for germination after the old stand has been cut. Fire should be kept out of the cut-over area. On the upper slopes, where for protective reasons clear-cutting can not be followed, the selection system is applicable, in which rather heavy cuttings should be made in order to open up the stand sufficiently for noble fir to take hold. Due to its superior qualities, noble fir should be favored more than any other species, with the exception of western white pine, in the upper portions of the lower-slope and in the lower portions of the upper-slope type.

GROWTH

It is probably in direct competition with a rather intolerant species such as Douglas fir that noble fir attains its best growth, both in diameter and in height.

Figure 1 illustrates graphically the diameter growth of noble fir, Douglas fir, and western hemlock in a mature stand, where the trees of all species averaged about 400 years of age. The results are for all the dominant trees on the tract.

Table I gives the average rate of diameter growth and height growth of noble fir in a mixed stand of Douglas fir and western hemlock at a elevation of from 3,000 to 3,500 feet on the west slope of the Cascade Mountains in northern Oregon.

TABLE I.—Average rate of diameter and height growth of noble fir, in mixed stand, on the west slope of Larch Mountain, Oreg.

Age	Average diameter at breast height	Average annual diameter growth in each decade	Average total height	Average annual height growth in each decade	Age	Average diameter at breast height	Average annual diameter growth in each decade	Average total height	Average annual height growth in each decade
Years	Inches	Inches	Feet	Feet	Years	Inches	Inches	Feet	Feet
10.....	0.0	4.0	130.....	25.0	0.14	116.1	0.51
20.....	3.1	0.31	12.0	0.80	140.....	26.3	.13	120.9	.48
30.....	6.0	.39	23.5	1.15	150.....	27.6	.13	125.4	.45
40.....	8.7	.27	38.4	1.49	160.....	28.8	.12	129.7	.43
50.....	11.1	.24	53.0	1.46	170.....	30.0	.12	133.8	.41
60.....	13.3	.22	65.5	1.25	180.....	31.1	.11	137.8	.40
70.....	15.3	.20	76.2	1.07	190.....	32.2	.11	141.7	.39
80.....	17.1	.18	85.2	.90	200.....	33.2	.10	145.5	.38
90.....	18.8	.17	92.8	.76	250.....	37.9	.09	162.2	.31
100.....	20.5	.17	99.5	.67	300.....	42.1	.08	175.8	.26
110.....	22.1	.16	105.5	.60	350.....	45.9	.07	187.3	.21
120.....	23.6	.15	111.0	.55	400.....	49.4	.07	196.3	.16

Table II gives the seedling height growth of noble fir grown under average conditions in the same locality as indicated in Table I.

Table III gives the average number of years required for noble fir seedlings to attain various heights.

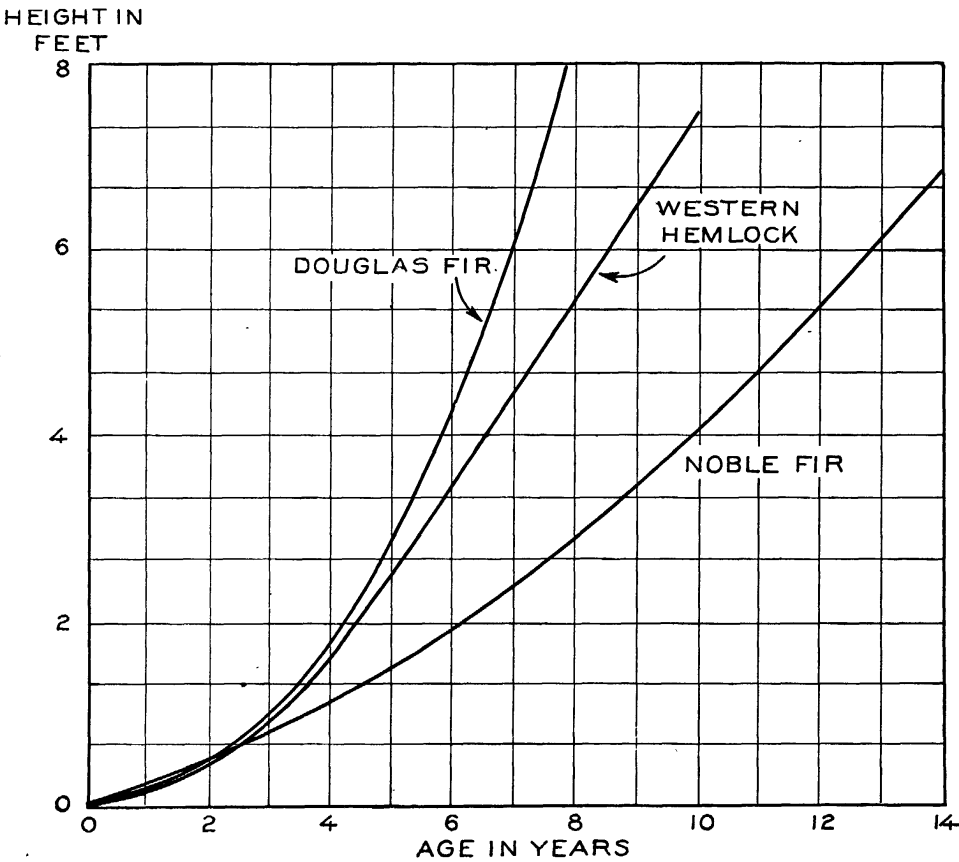


FIG. 2.—Height-growth curves for seedlings of noble fir, Douglas fir, and western hemlock.

Figure 2 illustrates graphically the seedling height growth of noble fir, Douglas fir, and western hemlock, in western Washington and Oregon.

TABLE II.—Seedling height growth of noble fir ^a

Age	Height	Current annual growth	Age	Height	Current annual growth
Years	Feet	Feet	Years	Feet	Feet
1			9	3.45	.55
2			10	4.04	.59
3	0.84	0.28	11	4.69	.65
4	1.16	.32	12	5.38	.69
5	1.53	.37	13	6.12	.74
6	1.94	.41	14	6.88	.76
7	2.39	.45	15	7.62	.74
8	2.90	.51	16	8.32	.70

^a Based on 282 measurements.

The seedling height growth of noble fir is slower than that of either Douglas fir or western hemlock. To attain breast height, 4.5 feet above the ground, it takes noble fir an average of 11 years, open-grown western hemlock 7 years, and Douglas fir 6 years.²

² MUNGER, T. T. THE GROWTH AND MANAGEMENT OF DOUGLAS FIR IN THE PACIFIC NORTHWEST. U. S. Dept. Agr., Forest Serv. Circ. 175, 27 p., illus. 1911.

TABLE III.—Average number of years required for noble fir seedlings to attain various heights, Larch Mountain, Oreg., 3,300 feet elevation

Height	Age	Height	Age	Height	Age	Height	Age
Feet	Years	Feet	Years	Feet	Years	Feet	Years
0.5-----	2	2.5-----	7	4.5-----	11	6.5-----	14
1.0-----	3	3.0-----	8	5.0-----	11	7.0-----	14
1.5-----	5	3.5-----	9	5.5-----	12	7.5-----	15
2.0-----	6	4.0-----	10	6.0-----	13	8.0-----	16

YIELD

Table IV gives the average contents per acre of standing trees of different species, no reduction being made for defect or breakage, for two plots differing essentially in the number of dominant trees forming the stand. Table V gives the average contents for 17¾ acres.

TABLE IV.—Average number of trees, average diameter, and average volume, by tree classes and species ^a

Plot and species	Dominant trees per acre	Diameter of average dominants	Total volume of dominants per acre	Suppressed trees 12 inches and more in diameter per acre	Volume of suppressed trees per acre	Total trees per acre with breast-height diameter of 12 inches or more	Total volume of all trees per acre
	Number	Inches	Bd. ft.	Number	Bd. ft.	Number	Bd. ft.
Plot 1—4 acres:							
Noble fir-----	8.50	45.6	51,887	1.00	1,125	9.50	53,012
Western hemlock-----	4.25	42.1	16,892	20.25	13,922	24.50	30,814
Douglas fir-----	12.25	42.6	41,111	1.50	1,892	13.75	43,003
Red cedar-----	.75	48.8	2,327	.75	384	1.50	2,711
Total-----	25.75		112,217	23.50	17,323	49.25	129,540
Average-----		43.7					
Plot 2—3 acres:							
Noble fir-----	8.33	55.0	79,667	1.33	536	9.66	80,203
Western hemlock-----	3.33	44.1	14,821	30.66	39,594	34.00	54,415
Douglas fir-----	4.00	48.5	19,508	.66	1,127	4.66	20,635
Amabilis fir-----				.33	267	.33	267
Total-----	15.66		113,966	33.00	41,524	48.65	155,520
Average-----		51.2					

^a Under "dominants" are included both dominants and codominants in the principal stand.

TABLE V.—Average yield per acre of mature noble fir, Douglas fir, and western hemlock, on Larch Mountain, Oreg.^a

Species	Trees per acre	Basal area	Diameter of average tree	Yield per acre
	Number	Sq. ft.	Inches	Bd. ft.
Noble fir-----	10	127	47.6	69,415
Douglas fir-----	7	66	42.0	22,268
Western hemlock-----	40	146	25.9	49,427
All species-----	57	339	30.4	141,110

^a Averages for 17¾ acres. Data are for trees 12 inches or more in diameter at breast height. Noble fir is utilized to a top diameter of 15 inches inside the bark; Douglas fir to 10 inches inside the bark; and hemlock to 8 inches inside the bark.

No data were obtained concerning the yield of young or second-growth noble fir stands in the course of this study.

The logging of noble fir of the extreme size found presents an enormous amount of waste caused mainly by breakage. This is unavoidable in many instances, although a great deal of care is exercised by the tree fallers in placing the trees in falling. In addition to the breakage, considerable defect was found, especially in the larger sizes, in some instances necessitating culling of the butt log. Many of these large trees were found to be hollow on the stump, the rot extending up from 10 to 30 feet. Ring shakes were prevalent, scarcely any of larger trees being free of them. It is estimated that the deduction for defect and breakage would amount to from 20 per cent to 30 or 40 per cent of the total standing contents of the trees as given in Table VI.

TABLE VI.—Comparative volumes of noble fir and associating species

Diameter at breast height	Volume			Diameter at breast height	Volume		
	Noble fir ^a	Douglas fir ^b	Western hemlock ^c		Noble fir ^a	Douglas fir ^b	Western hemlock ^c
Inches	Bd. ft.	Bd. ft.	Bd. ft.	Inches	Bd. ft.	Bd. ft.	Bd. ft.
30-----	2, 220	1, 250	1, 830	46-----	6, 200	4, 250	4, 890
32-----	2, 650	1, 450	2, 115	48-----	6, 840	4, 800	5, 255
34-----	3, 100	1, 690	2, 450	50-----	7, 500	5, 350	5, 595
36-----	3, 560	1, 950	2, 840	52-----	8, 180	5, 875	5, 920
38-----	4, 040	2, 275	3, 255	54-----	8, 900	6, 450	6, 240
40-----	4, 540	2, 700	3, 680	56-----	9, 630	7, 050	6, 560
42-----	5, 070	3, 120	4, 095	58-----	10, 400	7, 650	6, 880
44-----	5, 630	3, 650	4, 500	60-----	11, 170	8, 300	7, 200

^a Noble fir utilized to a 15-inch top diameter inside the bark.
^b Willamette Volume Table for Douglas fir. Trees utilized to a 10-inch top diameter inside the bark.
^c Western Washington Volume Table for western hemlock. Utilized to an 8-inch top diameter inside the bark.

VOLUME

Noble fir, due to its long cylindrical bole and thin bark, has a greater volume for its diameter at breast height than any of its associating species, with the possible exception of western white pine. In Table VI a comparison of the board-foot contents of trees of equal diameter of noble fir, Douglas fir, and western hemlock shows plainly the greater volume of noble fir. The bark rarely exceeds 2 inches in thickness, averaging about 1½ inches on the stump for the larger trees. Douglas fir, on the other hand, has bark from 4 to 8 inches in thickness for corresponding sizes. The bark of hemlock is not any thicker than that of noble fir, but its bole is not nearly so cylindrical and the average length runs considerably less, which accounts for the smaller volume of hemlock for equal diameters.

THE GENETICS OF SEX IN HEMP¹

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INTRODUCTION

In a recent paper (7)² the writer showed that the relative length of day and night exerts a profound influence on the development of both vegetative and floral parts of hemp. The results show that the length of time between planting and flowering in this species may vary from about 30 days to 90 days or more, depending on the relative length of day and night, other conditions being optimum for growth. The effect on the flowers was both quantitative and qualitative. When the period of daylight was long the plants were either pure carpellate or pure staminate, but when the period of daylight was short a number of intersex types appeared. Since the carpellate and the staminate flowers are normally produced on separate plants, such behavior is important because of its bearing on the mechanism of sex determination in this as well as in other dioecious species of plants.

There is no valid reason at present for doubting that environmental factors are largely responsible for the production of intersex types when hemp is grown in hothouses during the winter months. The results obtained by Schaffner (10, 11, 12) and by the writer (7) show quite clearly that individuals of either sex may produce flowers of the opposite sex when grown under such conditions. Sometimes such flowers are normal, but more often they are abnormal and sterile. The peculiarity of the case lies in the fact that some individual plants are affected much more than others grown under the same conditions and at the same time. In fact, some individuals do not produce flowers of the opposite sex under any conditions. The other extreme is reached in a case reported by the writer (7) in which a staminate-type plant matured several seeds. This is truly a reversal of the sexual function of the individual. This behavior, however, has led some botanists to suggest that sex in hemp is therefore nonmendelian in nature and can not be explained by a chromosomal mechanism. With such a suggestion the writer can not fully agree. The mere fact that the normal sexual expression of the individual is modified by certain factors does not prove that there is not a definite mechanism which is responsible for its development. It is merely the manifestation of the effect on the mechanism.

The point at issue, then, is whether or not the phenomenon of sex in hemp can be explained on a genetic basis. It would seem that the solution of this problem can be brought about only by a series of

¹ Received for publication March 20, 1925; issued January, 1926. The results reported in this paper were obtained at the Laboratory of Plant Genetics, Bussey Institution of Harvard University, and completed in the United States Department of Agriculture.

² Reference is made by number (italic) to "Literature cited," p. 942.

carefully planned breeding experiments in which the environmental factors are under the control of the operator. Published results of such experiments are conspicuous by their absence, and the presentation of data on this subject is the purpose of this paper.

PRELIMINARY OBSERVATIONS

Among plats of several varieties of hemp grown at the Bussey Institution of Harvard University during the summer and winter of 1921-22, the writer observed that in addition to the appearance of the usual intersex types in the hothouse during the winter there appeared a number of monoecious plants in the summer plat of the Simple Leaf variety. Almost without exception these individuals had the carpellate vegetative development and had been first classed as pure carpellate plants. The staminate buds were first observed when the seeds were about half mature. As a rule they were not restricted to any particular part of the plant, but were fairly well distributed among the seed-producing branches. Practically all staminate flowers thus produced were identical in appearance with those produced by pure staminate plants. Inasmuch as this plat of hemp was grown during the summer season, when the occurrence of "sex reversal" is rare, the production of functional flowers of both sexes on single individuals furnishes a starting point for breeding experiments to determine the extent to which such a phenomenon is hereditary. At first sight it may seem that such a case of "sex reversal" is different from that which occurs so commonly in hothouses during the winter. The two cases are fundamentally the same, however, and differ only in degree. The only difference lies in the fact that the abnormal flowers so common during the winter were absent from the monoecious Simple Leaf plants. The real question to be answered in either case is, what is the difference between the pollen produced by a carpellate type and that produced by a pure staminate type?

The sex ratios observed in the preliminary experiments (Table I) show a few peculiarities of minor importance. The most common condition seems to be about equal numbers of carpellate and staminate types. The minor fluctuations from this may be due to differential germination, death, or sampling of the seed. The sex ratio in the Simple Leaf variety, however, may be due to other causes not yet known. Since this variety arose as a mutation, it is suggested that its sexual state may be genetically different from that of the other varieties tested.

TABLE I.—Sex ratios in different varieties of hemp

Variety	Environment	Number	Sex ratio	Notes ^a
Chington	Field plat	472	105 : 100	No intersex types.
Kejo	do	136	106 : 100	Do.
Tochymington	do	341	130 : 100	1 intersex.
Simple Leaf	do	401	212 : 100	56 monoecious. ^b
Chington	Hothouse in summer	382	104 : 100	1 intersex.
Do	Hothouse in December-January	124	100 : 100	44 intersexes.
Do	do	103	93 : 100	17 intersexes.

^a All plants which produced abnormal flowers are, for convenience, classes as intersexes.
^b These plants were classed as carpellate in the sex ratio.

The intersex types (Table I) which occurred in the Chington variety were of various kinds, corresponding in general with those described by Schaffner (9, 10, 11, 12). In this connection it may be well to recall the fact already indicated that the growing of hemp in a hothouse does not, in itself, tend to bring about "sex reversal." Only one of the 382 Chington plants which were grown in a hothouse during the summer produced any flowers of the opposite sex. The proportion in the open plats was about 1 in 800. Both of these proportions are of relatively little significance, except that they demonstrate that "sex reversal" may occur during the long daylight period of summer. Although it is quite clear that relative length of day and night is an important factor in bringing about "sex reversal," it is not the only factor causing such changes.

BREEDING EXPERIMENTS

The general plan of the experiments was to make self and cross pollinations among the extreme intersex types in order to ascertain whether the tendency toward sex reversal was inherited. When the staminate buds were observed growing on what had been previously classed as pure carpellate Simple Leaf plants, the plats were visited several times daily, and when the buds were nearly ready to open each was removed, carefully brushed to remove foreign pollen and placed in a stoppered vial. These were kept in the laboratory until the anthers opened and liberated the pollen. This pollen was then transferred to stigmas of carpellate flowers which had been bagged for some time previously. Both pure carpellate and monoecious individuals were used. Three types of pollinations were made, namely, self-pollination, pollination of a pure carpellate with pollen from an intersex type, and cross-pollination of Chington stock plants as a check. From this series of pollinations a total of 326 seeds was obtained. When these seeds were planted in the greenhouse the results shown in Table II were obtained.

TABLE II.—*First generation of crosses between intersex types*

Variety	Number of seed planted	Carpellate	Staminate	Total
Simple Leaf intersex selfed.....	100	71	0	71
Simple Leaf carpellate × Simple Leaf intersex.....	64	56	0	56
Simple Leaf carpellate × Chington intersex.....	37	31	3	34
Chington carpellate × Chington staminate.....	148	46	42	88

It is at once obvious that there is a difference between the sex ratio of the check and that of each of the three other lots. The 88 plants in the check plat happened to give almost exactly a 1:1 ratio of the sexes, but of the other 161 plants, representing 3 series of pollinations, all except 3 were carpellate. The appearance of the 3 staminate individuals among the progeny of the Simple Leaf carpellate × Chington intersex cross is not in accord with the results of the other crosses.

The probable cause of this, however, is not difficult to find. The enormous amount of pollen which is produced by a single staminate hemp plant makes the elimination of contamination very difficult;

and although the work was conducted with great care there is a possibility nevertheless that a few stray pollen grains gained admission to the bagged branches of the Simple Leaf plants.

The 158 first-generation carpellate plants were grown in the hot-house during the winter months, and under these conditions several of them produced some staminate flowers; otherwise all were typically carpellate. The small amount of pollen which they produced was all used to make self-pollinations. As no staminate plants were used in the same house during the growth period, the chance of possible fertilization by pollen from a staminate plant was eliminated. It seems reasonable to assume, therefore, that any accidental pollination which might take place through carelessness would be a cross between two carpellate-type plants.

The number of staminate flowers available as sources of pollen was very small, and consequently the number of selfings which could be made was small. No cross-pollinations were attempted. The total number of seeds obtained was 193, many of which were small and shriveled. When these were planted the results shown in Table III were obtained.

TABLE III.—Results from self-pollinating carpellate type intersex

Cross	Seeds planted	Number dying young	Number of carpellate	Number of staminate
M-4 self.....	66	2	6	0
S-8 self.....	30	0	1	0
S-1 self.....	6	0	5	0
M-3 self.....	25	0	6	0
A-10 self.....	66	12	28	0
Total.....	193	14	46	0

The very poor germination of the seed is a most disappointing feature of these results. There is evidently a marked difference between the strains in this respect, but whether this is due to inherent characters or to other causes is a question which can not be answered from the data available. But of those which did germinate and grow to the flowering stage all were carpellate. Of course, these results do not prove that the progeny obtained by selfing a carpellate type intersex will all be of the carpellate type, but so far as they go they substantiate the results obtained in the previous generation.

Up to this point results obtained by selfing carpellate intersex types have been presented, and now it might be well to consider the possibilities of self-pollinating a staminate-type intersex. By this is meant a plant which is typically staminate until after the first few flowers have opened and then begins to produce also some flowers of the carpellate type. If this can be done it may yield some valuable information concerning the inherent differences between the carpellate and staminate types. For several reasons, such a plan holds forth relatively few chances of success. In the first place, the development of functional carpellate flowers on the staminate type intersex is much rarer than is the production of viable pollen by carpellate type intersexes; and, secondly, the inherent difference in the length of life of the two types makes it difficult to coax the staminate plant

to remain green long enough to properly mature seed. It will be recalled that the staminate type dies soon after the maturity of the pollen, while the carpellate type remains green and vigorous until the seed is mature.

By carefully regulating the relative length of day and night, and making drastic mutilations, the writer has succeeded in causing hemp which was at first typically staminate to produce functional carpellate flowers. The effects of the manipulations on the vegetative development were so great, however, as to make the plants very small and weak. It was, therefore, not advisable to bag them, and the seeds which they matured may have been the result of either cross or self-pollination. Only 11 seeds matured, and of these only 4 grew to the flowering stage. The sex distribution was 3 staminate and 1 carpellate. Of course, these results are entirely too meager to warrant an extended discussion. The only significant fact brought out is that the staminate type carries the potentialities of the opposite sex type.

DISCUSSION

In the foregoing presentation of data, relatively little attention was given to the details of "sex reversal" which occurred during the winter, for the simple reason that they do not directly concern the immediate problem. The question to be answered concerns the mechanism of sex determination in hemp and not the effect of environmental or other factors upon this mechanism. When this much has been answered, then the time will be ripe for further inquiry into the details of each individual case of sexual modification.

The misinterpretation of the geneticist's conception of sex in dioecious plants has thrown some botanists into a chaos from which they have not yet emerged. It has been maintained that because a plant of one sex type develops some flowers of the opposite sex type, genetic factors are not concerned, and any question of homozygousness or heterozygousness is automatically ruled out. To substantiate such contentions abundant evidence is brought forth to prove that either sex type may produce flowers of the other type. With the facts shown by such data the writer agrees fully. The facts are fairly clear cut. There is a distinct vegetative dimorphism which is the same for both the sexual forms which remain pure and those which sooner or later show various sexual modifications. Under the ordinary cultural conditions found in the field these types occur in approximately a 1:1 ratio. The production of normal flowers of the opposite sex under such conditions, however, has been observed frequently. Among plants grown in the hothouse during the winter the sex ratio is approximately 1 : 1, but usually a large proportion of the plants develop flowers of the opposite sex. These flowers are usually abnormal. Such behavior is critical evidence that the normal development is being modified by environmental factors, but certainly should not be interpreted as meaning that certain genes are not responsible for the normal development of the character. There is abundant evidence from the well-known genetics of certain vegetative characters to show that the environment in which they develop may have such an effect as to prevent their expression at all. As Emerson (5) has pointed out, the mere fact that the expression of one character is influenced more by environment than is that of some

other characters does not signify that the genetics of the case is any less important.

The key to the situation is evidently to be found in the mechanism which controls the sex ratio under normal cultural conditions. The simple assumption that one sex is homozygous and the other heterozygous would explain the occurrence of the 1:1 sex ratio. A cross between such types is expected to produce equal numbers of each type. The data presented in Tables II and III indicate that the carpellate type is the homozygous one. Then, the carpellate form might be represented by the symbols XX and the staminate type by the symbols XY. The selfing of a carpellate type or crossing with another carpellate-type plant would be expected to produce only carpellate-type individuals. The selfing of a staminate-type individual would be expected to give rise to carpellate, staminate, and YY individuals in the ratio 1:2:1. The nature of the YY form, if such exists, is a question on which data are lacking at present. Otherwise, the data thus far obtained can be explained on such a provisional hypothesis. It is not to be assumed, however, that the condition is as simple as such a hypothesis might lead one to believe. The data available concerning the progeny of a selfed staminate-type plant consists of only 4 individuals, of which 3 were staminate and 1 was carpellate. This is entirely too small a number of individuals to be of any significance and can be of use only in suggesting that the staminate type is heterozygous for the sex factors. This is a point which must be cleared up by further investigation.

If the staminate type is heterogametic, it may be assumed that there exists a chromosome difference between the two kinds of gametes produced. Cytological investigations, however, have failed to show any such differences. The investigations of Strasburger (14) and of the writer (8) have shown that there is apparently nothing in the chromosome complex of hemp which can be identified as a sex chromosome. The 20 pairs of chromosomes are so nearly the same size and shape that it is not possible to follow them individually through the stages of cell division. But of course this evidence does not preclude the possibility that one pair of chromosomes is carrying the sex genes. There is no reason why the chromosome which carries the genes for sex should have a hook on one end or be larger than any of the others. This condition does exist in some animals and plants, but it does not follow that there is a correlation between the presence of sex genes and size or shape of the chromosomes which carry them.

During recent years a vigorous search for sex chromosomes in dioecious species of plants has been in progress. The results, positive in many cases, indicate that the male sex is heterogametic. Such a condition being in accord with the results obtained in breeding experiments with hemp, it seems desirable to mention the outstanding cases.

The presence of sex chromosomes in *Lychnis dioica* has been shown by Winge (15). The female plants contain 22+XX and the males 22+XY. This condition has been confirmed by Blackburn (1), who finds the diploid number to be 24, of which 2 are larger than the other 11 pairs. In the staminate plants, one of the pair differed from the other in respect to both length and shape. The interesting

results of breeding experiments reported by Correns (2, 3), and the case of sex-linked inheritance of the XY type in this species reported by Shull (13), are in accord with the results of these cytological investigations. The presence of a visible chromosome difference in *Humulus lupulus* and in *H. japonicus* has also been shown by Winge (15). In both species the staminate type is heterogametic. So far as the writer is aware, there are no published results of breeding experiments to substantiate this finding.

Recently Kihara and Ono (6) have reported a peculiar chromosome condition in *Rumex acetosa*. The carpellate form contains 2 large M chromosomes and 12 autosomes. The staminate form contains 12 autosomes and 1 large "tripartite," which consists of a large M chromosome and two smaller elements. It is assumed that the large M is the X chromosome and the smaller elements the Y chromosome. Such a condition is in line with the results of Corren's (4) breeding experiments, from which he inferred that two kinds of pollen were being produced.

The evidence obtained from these sources shows that sex chromosomes exist in at least some of the dioecious species of plants. A fact of still greater importance is that both the genetic and cytological evidence points to the heterozygousness of the male form. It may seem, therefore, that a simple and general hypothesis should explain the mechanism of sex determination in such dioecious species. So little is known of the genetics of each species, however, that it seems to the writer that the accumulation of more genetic evidence should be awaited before a general hypothesis is formulated. For this reason the writer wishes to restrict to hemp his suggestions concerning sex in plants.

It has been repeatedly maintained during recent years that sex determination and segregation in dioecious species of plants has nothing to do with the segregation of the chromosomes, because sex is not determined until long after the reduction division and fertilization have taken place. Let us examine the evidence for such a contention in the case of hemp. It is not possible to distinguish between the carpellate and the staminate plants before the vegetative differences are developed or the flower buds appear. That is, during the early stages of growth all the plants appear to be the same. Is it to be assumed that the sex of such plants is not determined until just prior to the appearance of the flower buds? Is it to be assumed that in a plat of 10-day-old hemp the plants are neither staminate nor carpellate? The fact that it is impossible to distinguish the sexes one from the other at this stage of their life is not critical evidence that the sexual type has not been determined. If it could be shown, for instance, that in a plat of 1,000 plants all could be caused to develop into the carpellate type, there would be strong reason for believing that the sex of the individual is determined some time after the seed has germinated. But man has been unable to do this sort of thing, no matter what methods were used. Of course it has been shown repeatedly that as high as 90 per cent of plants in certain plats developed flowers of the opposite sex. Whether these flowers were the same genetically as those produced by the normal staminate or carpellate type is the really critical evidence which is lacking for these numerous cases. And until more of such evidence is available the

problem of sex determination in hemp as well as in other dioecious species of plants should be regarded with an open mind. The problem is so intricate that it probably will be fully solved only through the close cooperation between the geneticist and the physiologist.

SUMMARY

There is a distinct vegetative and sexual dimorphism in hemp. The vegetative differences are the same for those plants which remain sexually pure and those which later produce some flowers of the opposite sex.

The two sex types occur in approximately at a 1 : 1 ratio.

Under certain conditions both sex types produce flowers of the opposite sex. The most decisive factor in bringing this change about is the relative length of day and night.

When staminate and carpellate individuals are crossed, the progeny consists of staminate and carpellate in equal numbers. When the carpellate is selfed or pollinated with pollen from another carpellate type individual, the progeny are all carpellate. The seeds produced on a staminate plant give rise to both sex types.

It is maintained that sex determination in hemp can be explained on a genetic basis, and that the inheritance is of the XY type.

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EFFECTS OF FEEDING ANIMALS WITH TRICHINOUS MEAT CONTAINING NONVIALE TRICHINAE¹

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INTRODUCTION

In the extensive literature on trichinosis there appears to be no records of experiments dealing primarily with the effects produced by the ingestion of meat containing nonviable trichinae. Various workers have assumed, without adequate experimental evidence, that meat containing nonviable trichinae is innocuous in so far as the production of any of the symptoms that characterize trichinosis is concerned. Flury in 1913,² found that apart from the mechanical injuries caused by living trichinae in the host animal, the products formed as a result of their metabolic activities coincident with their growth and development in the host, as well as the products formed as a result of the degeneration of the muscles which they invade, are highly toxic to animals when injected parenterally. Flury, in fact, has explained the entire symptom complex of trichinosis, such as the gastric and intestinal disturbances, vomiting, local irritation, muscle stiffness, capillary hemorrhages, edema, blood changes, fever, and respiratory difficulties, as the result of (1) the toxic substances produced by the parasites, and (2) the degeneration products of the muscles which they invade. His careful studies, involving numerous experiments with various extracts of trichinous muscles, as well as the evidence furnished by various investigators regarding the toxicity of nematodes in general, naturally suggest the possibility that dead trichinae may also be injurious because of the liberation of possible toxic products as a result of their digestion, and that the ingestion of trichinous meat containing nonviable trichinae may give rise to at least some of the symptoms of trichinosis or to other unpleasant symptoms.

In order to determine the effects produced on animals by meat containing nonviable trichinae, several experiments were performed in which such meat was used. On account of their marked susceptibility to trichinosis, rats were chosen for the tests. Rats are likely to show severe symptoms not only after the parasites become established in the muscles but also during the intestinal stage, and usually die at this early stage of the disease even when they are fed very small quantities of heavily infested trichinous meat. Since rats frequently die of trichinosis before the larvae have begun to migrate, it may be assumed that death is due, in all probability, to a severe intoxication, coincident with the rapid growth and development of the parasites in the intestine, other probable contributory

¹ Received for publication June 19, 1925; issued January, 1926.

² FLURY, F. BEITRÄGE ZUR CHEMIE UND TOXIKOLOGIE DER TRICHINEN. Arch. Expt. Path. u. Pharmacol. 73: 164-213, illus. 1913.

factors being the effects of the severe irritation to the intestinal mucosa as a result of the rather deep penetration of the worms into the intestinal wall. If toxic substances are present in cooked and refrigerated trichinous meat in a state in which they are still capable of deleteriously affecting susceptible animals, the evidence of such toxicity should be demonstrable by feeding the meat to rats.

EXPERIMENTS

SERIES I

Meat from a trichinous rabbit, containing viable encapsuled worms, was gradually heated in water until the latter began to boil. Liberal portions of this meat were fed to each of six rats on August 16, 18, and 19, 1924. The animals devoured it readily. No symptoms developed as a result of the experiment, the animals continuing to eat the usual oat ration and exhibiting their normal activities for several months.

SERIES II

Trichinous pork that was very heavily infested was refrigerated for a period of 25 days at a temperature below 5° F., a procedure which is destructive to the vitality of trichinae. Liberal portions of this pork selected from parts of the carcass that were known to be heavily parasitized, namely, the diaphragm and the intercostal muscles, were fed to a series of 12 rats on December 3, 4, 6, 9, and 12, 1924. The rats showed no evidence of discomfort. They were kept alive on their usual ration for several months after the experimental feedings and continued throughout this period in apparent good health.

SERIES III

Liberal portions of cooked trichinous pork from a heavily infested hog were fed to 6 rats on January 10, 13, 19, 21, and 24, 1925. A sufficient quantity of meat was given each time to last from two to three days. All the meat given was consumed. No ill effects were observed, the animals continuing in good health for about two months after the experiments. Subsequently they were used in other experiments.

SERIES IV

The same animals that were used in Series III were fed trichinous pork refrigerated as described in connection with Series II. The rats were fed as follows: February 9, 1925, meat enough to last three days; March 6, 1925, enough to last two days; March 14, 1925, enough to last three days. The animals consumed all the meat that they were given and showed no ill effects. They were apparently in perfect health on May 25, 1925, on which date they were used in other experiments.

SERIES V

Three dogs and three cats were fed liberal portions of refrigerated trichinous pork, prepared in accordance with the method described in connection with the experiments in Series II. Sufficient meat was given each time to last from two to three days, no other feed being given during that period. The animals were fed on the follow-

ing dates: January 2, 4, 7, and 9, 1925. No ill effects were observed in these animals during the period of several weeks in which they were under observation.

DISCUSSION

On the basis of experiments described in this paper, in which relatively large quantities of meat containing nonviable trichinae were fed to animals with consistently negative results, it may be concluded that neither the dead trichinae themselves nor the muscles in which they were are encysted are injurious when ingested by animals susceptible to trichinosis, namely, rats, dogs, and cats. There is no reason to doubt the view that susceptible animals other than those used in these experiments, including human beings, would likewise be unaffected as a result of the ingestion of cooked trichinous meat or of trichinous meat refrigerated sufficiently to destroy the vitality of trichinae. If toxic substances are present in encapsuled trichinae and in the muscles in which they are lodged, these substances are either destroyed in the process of cooking and refrigeration or are broken down or neutralized in the course of digestion before they can exert deleterious effects on test animals.

The experiments described in this paper are in agreement with the commonly accepted view that after trichinous meat has been cooked sufficiently, refrigerated sufficiently, or treated by some other method known to destroy the vitality of trichinae, it is no longer capable of producing the symptoms characteristic of trichinosis. These experiments are useful in supplying an experimental verification of a view that has been commonly accepted on the basis of belief rather than on the basis of experimental proof.

SUMMARY

Rats, dogs, and cats were found to be tolerant of heavy doses of meat containing nonviable trichinae, exhibiting no ill effects as a result of repeated ingestion of such meat. These observations are in harmony with the generally accepted view that pork in which trichinae have been destroyed by cooking or by some other method known to devitalize trichinae will not produce any of the symptoms characteristic of trichinosis or other harmful effects.

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LIMITATION OF STUDENT'S METHOD WHEN APPLIED
TO FERTILIZER EXPERIMENTS¹

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The advantages of Student's method for interpreting paired experiments are fully recognized by many agronomists largely because of the fact that this method brings within the range of statistical inquiry many crop and fertilizer tests the small-sample results of which furnish insufficient variability to enable one to judge of the significance or certainty of the results by the application of the usual probable-error formulas.

It has been pointed out by Love and Brunson² (8) that biometrical methods must be used with great care, that experimental data must be wisely and judiciously interpreted if the conclusions reached are to be of permanent value, and that Student's method is no exception to this rule. These same writers have also suggested that this method can be successfully applied only when the paired observations are made under similar conditions, or when the members of each pair are exactly comparable.

This present paper is presented with a view to illustrating, briefly, some of the limitations of Student's method when used in the interpretation of fertilizer results, and to calling attention to some precautions that should be taken in arriving at any mathematical expression of the significance or nonsignificance of one fertilizer treatment as compared with another.

Let the data in Table I be assumed for a pair of fertilizer tests on corn, obtained on two differently fertilized plots, A and B, belonging to the same series of tests:

TABLE I.—*Fertilizer tests on corn on differently fertilized plots*

Year	Plot A		Plot B	
	Yield per acre	Increase effected per acre	Yield per acre	Increase effected per acre
	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>
1900.....	77.20	14	75.60	14
1901.....	53.50	12	49.70	11
1902.....	66.10	14	56.50	14
1903.....	65.80	10	63.80	11
1904.....	52.40	9	42.40	8
1905.....	57.30	13	47.70	14
Mean.....	62.05	12	55.95	12

¹ Received for publication Feb. 20, 1925; issued January, 1926.
² Reference is made by number (*italic*) to "Literature cited," p. 956.

When Student's method is applied to the paired *yields* a conclusion is reached showing the odds to be 195 to 1 that the fertilizer applied to plot A is more effective than the fertilizer applied to plot B. This can be true only when the natural soil productivity is a constant. However, if soil heterogeneity prevails to the extent that plot A represents a higher natural productivity than that represented by plot B, and this establishing a high degree of parallelism between the paired yields owing to the fact that yield A is always higher than B, in all probability the odds of 195 to 1 carry but little or no significance as regards the fertilizer applied to plot A.

Now let it be further assumed that the figures in the second column under A and B represent the actual increases effected in each case, these being entirely within the range of probability. In comparing the paired *gains*, either by a simple comparison of the data or by applying Student's method, it becomes plainly evident that no significance can be attached to the higher yields obtained on plot A, which are higher because of the difference in inherent productivity. It seems an absolute certainty that fertilizer A is not the more effective fertilizer, especially when cognizance is taken of the fact that the average gain effected by the one fertilizer is equal to the average gain effected by the other.

The foregoing illustration, though hypothetical, raises the question as to how Student's method should be applied to fertilizer results. Should the actual yields or the gains effected be paired?

It is a fact generally known that the soil constituting the plots of a series of tests in an experiment, though it be of the same type, usually varies more or less in its producing power on a given area, thus introducing the factor of soil heterogeneity in the interpretation of results. This condition, together with the fact that in but a few of the long-time fertility experiments already established are any of the fertilizer treatments repeated, makes it highly probable that the pairing of actual yields by Student's method may not establish certainty that one fertilizer treatment is more effective than another. This point is well illustrated by the published 18-year results obtained with wheat on plots Nos. 11 and 30 in the potato-wheat-clover rotation test at Wooster, Ohio, which follow (1, 3, 4, and Table II).

TABLE II.—*Student's method applied to wheat yields obtained in potato-wheat-clover rotation at Wooster, Ohio*

Year	Actual yields		Difference in yields, No. 11 over No. 30	Deviation from 5 68 (D)	D ²	Gains effected		Difference in gains, No. 11 over No. 30	Deviation from 1.88 (D)	D ²
	Plot No. 11 ^a	Plot No. 30 ^b				Plot No. 11	Plot No. 30			
	<i>Bush.</i>	<i>Bush.</i>	<i>Bush.</i>			<i>Bush.</i>	<i>Bush.</i>	<i>Bush.</i>		
1895.....	16.25	9.92	6.33	0.65	0.42	8.03	3.64	4.39	2.51	6.30
1896.....	15.20	5.66	9.54	3.86	14.90	8.14	— .42	8.56	6.68	44.62
1897.....	45.00	34.67	10.33	4.65	21.62	8.44	.72	7.72	5.84	34.11
1898.....	33.25	35.08	—1.83	7.51	56.40	9.36	10.39	—1.03	2.91	8.47
1899.....	39.00	30.50	8.50	2.82	7.95	9.75	6.67	3.08	1.20	1.44
1900.....	46.91	38.83	8.08	2.40	5.76	2.33	5.83	—3.50	5.38	28.94
1901.....	29.17	29.00	.17	5.51	30.36	3.56	6.59	—3.03	4.91	24.11
1902.....	55.00	41.52	13.48	7.80	60.84	16.47	10.99	5.48	3.60	12.96
1903.....	42.33	34.17	8.16	2.48	6.15	11.08	11.25	— .17	2.05	4.20
1904.....	35.96	31.54	4.42	1.26	1.59	14.89	9.79	5.10	3.22	10.37
1905.....	37.00	29.71	7.29	1.61	2.59	12.83	10.79	2.04	.16	.03
1906.....	52.33	43.50	8.83	3.15	9.92	6.02	5.92	.10	1.78	3.17
1907.....	42.75	37.42	5.33	.35	.12	8.03	2.70	5.33	3.45	11.90
1908.....	50.42	44.37	6.05	.37	.14	11.15	13.29	—2.14	4.02	16.16
1909.....	43.54	42.12	1.42	4.26	18.15	2.40	7.80	—5.40	7.28	53.00
1910.....	34.12	34.00	.12	5.56	30.91	11.44	8.30	3.14	1.26	1.59
1911.....	25.83	30.83	—5.00	10.68	114.06	12.24	13.61	—1.37	3.25	10.56
1912.....	18.04	6.92	11.12	5.44	29.59	6.52	.91	5.61	3.73	13.91
Mean.....	36.80	31.10	5.68	-----	22.86 $\sigma=4.80$	9.04	7.15	1.88	-----	15.88 $\sigma=3.98$

$Z=1.18$ (5, p. 416).
 $P=\pm 0.9999+$.
Odds=Over 10,000 to 1.

$Z=0.471$.
 $P=\pm 0.9635$.
Odds=26 to 1.

^a Fertilizer treatment consists of 120 pounds of nitrate of soda, 50 pounds of dried blood, 160 pounds of acid phosphate, 100 pounds of muriate of potash, applied to wheat; and 80 pounds of nitrate of soda, 160 pounds of acid phosphate, and 100 pounds of muriate of potash, applied for potatoes.
^b Fertilizer treatment consists of 8 tons of manure applied for potatoes.

Here it is shown that, because of the striking parallelism of the paired yields, the fertilizer treatment given to plot No. 11 is much superior to that given plot No. 30, this certainty being expressed by odds far exceeding 10,000 to 1. However, when the actual gains effected are paired, the parallelism is broken at seven different points, thus presenting in graphic form a more or less crisscross effect. Consequently, the odds are not sufficiently high to establish the fact that there is any important difference in the two treatments, odds 30 to 1 being accepted as the mathematical expression for establishing statistical significance.

In some cases when paired yields are subjected to Student's method for interpretation, odds indicate no degree of certainty that one fertilizer is better than another; but when the paired *gains* are subjected to the same test, odds are sufficiently high to enable one to come to quite a different conclusion. To illustrate this point, the published results obtained with corn for 20 years on plots Nos. 17 and 26 in the five-year rotation at Wooster have been selected, as follows (1, 3, 4, and Table III):

TABLE III.—*Student's method applied to corn yields in five-year rotation at Wooster, Ohio*

Year	Actual yields		Difference in yields No. 17 over No. 26	Deviation from .78 (D)	D ²	Gains effected		Difference in gains No. 17 over No. 26	Deviation from 4.31 (D)	D ²
	Plot No. 17 ^a	Plot No. 26 ^b				Plot No. 17	Plot No. 26			
1894.....	<i>Bush.</i> 20.50	<i>Bush.</i> 18.32	<i>Bush.</i> 2.18	1.40	1.96	<i>Bush.</i> -0.62	<i>Bush.</i> 1.80	<i>Bush.</i> -2.42	6.73	45.29
1895.....										
1896.....	56.89	59.43	-2.54	3.32	11.02	5.80	8.74	-2.94	7.25	52.56
1897.....	31.79	42.86	-11.07	11.85	140.42	13.11	9.48	3.63	.68	.46
1898.....	33.93	38.75	-4.82	5.60	31.36	8.06	7.24	.82	3.49	12.18
1899.....	40.50	34.50	6.00	5.22	27.25	18.81	10.36	8.45	4.14	17.14
1900.....	48.68	41.32	7.36	6.58	43.30	21.35	10.75	10.60	6.29	39.56
1901.....	60.64	63.14	-2.50	3.28	10.76	19.35	28.64	-9.29	13.60	184.96
1902.....	75.57	89.18	-13.61	14.39	207.07	23.25	22.92	.33	3.98	15.84
1903.....	22.32	23.43	-1.11	1.89	3.57	19.67	13.83	5.84	1.53	2.34
1904.....	40.04	34.46	5.58	4.80	23.04	22.95	19.40	3.55	.76	.57
1905.....	58.64	49.18	9.46	8.68	75.34	20.86	18.25	2.61	1.70	2.89
1906.....	63.32	64.54	-1.22	2.00	4.00	28.12	29.33	-1.21	5.52	30.47
1907.....	48.29	64.57	-16.28	17.06	291.04	12.98	17.56	-4.58	8.89	79.03
1908.....	44.96	43.64	1.32	.54	.29	20.16	10.51	9.65	5.34	28.52
1909.....	45.07	33.67	11.40	10.62	112.78	25.29	14.11	11.18	6.87	47.20
1910.....	17.07	12.03	5.04	4.26	18.15	13.09	3.19	9.90	5.59	31.25
1911.....	71.75	69.10	2.65	1.87	3.50	35.28	25.02	10.26	5.95	35.40
1912.....										
1913.....	42.50	26.25	16.25	15.47	239.32	29.87	8.58	21.29	16.98	288.32
Mean	45.70	44.90	+0.78	-----	69.12 $\sigma=8.31$	18.74	14.43	+4.31	-----	M=50.78 $\sigma=7.12$

 $Z=0.0939.$ $P=\text{less than } \pm 0.6575.$

Odds=less than 2 to 1.

 $Z=0.61.$ $P=\pm 0.9886.$

Odds=87 to 1.

^a Fertilizer treatment consists of 60 pounds of nitrate of soda, 25 pounds of dried blood, 160 pounds of acid phosphate, 100 pounds of muriate of potash, applied to wheat; and 80 pounds of nitrate of soda, 160 pounds of acid phosphate, and 80 pounds of muriate of potash, applied to corn and oats.

^b Fertilizer treatment consists of 50 pounds of dried blood on wheat; 120 pounds, 240 pounds, and 240 pounds of nitrate of soda, applied on wheat, corn, and oats, respectively; bone meal equivalent to 160 pounds, 80 pounds, and 80 pounds of acid phosphate, applied to wheat, corn, and oats, respectively; and 100 pounds, 80 pounds, and 80 pounds of muriate of potash, applied to wheat, corn, and oats, respectively.

In this case a parallelistic analysis of the yields shows that the one fertilizer is practically as efficient as the other, but when Student's method is applied to the gains obtained, the odds are 87 to 1 that the fertilizer applied to plot No. 17 is more effective than that applied to plot No. 26.

It may be of interest to cite four other cases to illustrate how a conclusion reached by applying Student's method to paired yields may be reversed when the parallelisms of the corresponding gains are studied (Table IV).

TABLE IV.—*Student's method applied to paired yields and to the corresponding paired gains, in experiments at Wooster, Ohio*

Crop	Experiment and period of test	Plots compared	Fertilizer treatment	Interpretation of fertilizer results			
				Student's method applied to paired yields		Student's method applied to paired gains	
				Average yields	Statistical significance	Average gains	Statistical significance
Corn.....	Manure test, 3-year rotation, 1897 to 1913, inclusive.	{ 2	Yard manure reenforced with rock phosphate.	Bush. 62.60	{ Odds less than 2 to 1; acid phosphate as good as rock phosphate.	Bush. 26.50	{ Odds greater than 10,000 to 1; acid phosphate is better than rock phosphate.
		{ 5	Yard manure reenforced with acid phosphate.	61.80		31.50	
Wheat.....	Manure test, 3-year rotation, 1898 to 1913, inclusive.	{ 2	-----do-----	24.50	{ Odds 18 to 1; no significance.	12.48	{ Odds 3,332 to 1; acid phosphate is better than rock phosphate.
		{ 6	Stall manure reenforced with acid phosphate.	25.40		14.85	
Do.....	Manure test, 3-year rotation, 1898 to 1913, inclusive.	{ 3	Stall manure reenforced with rock phosphate.	25.89	{ Odds about 1 to 1; no significance	15.67	{ Odds 63 to 1; acid phosphate is better than rock phosphate.
		{ 18	Manure, 8 tons on each corn and wheat.	25.85		14.32	
Corn.....	Five-year rotation, 1895 to 1906, inclusive.	{ 23	Complete chemical fertilizer on corn oats, wheat.	39.94	{ Odds about 2 to 1; no significance	13.89	{ Odds 399 to 1; manure is the more efficient fertilizer.
				38.95		10.93	

In many experiments and probably in the majority of specific instances it does not matter which parallelism is used in the interpretation of results, since the application of Student's method to either will establish significance or nonsignificance in the same direction. In the foregoing examples it has been shown that the application of Student's method to paired gains may give odds that will not support the conclusion drawn when the method is applied to the corresponding pairs of yields, this being the way the method has been generally applied (2; 6, p. 10; 7, p. 22; 8; 9; 10; 11, p. 6). Moreover, when the interpretation of fertilizer results is based on the parallelism of yields it may even be shown that fertilizer treatment X, for example, is more effective than fertilizer treatment Y; but that when the interpretation of the results is based on the corresponding pairs of gains effected odds may indicate just about as high a degree of certainty that fertilizer treatment Y is the more effective. This may be illustrated by the published results obtained with wheat on plots Nos. 15 and 32 in the potato-wheat-clover rotation at Wooster for the period 1895 to 1912, inclusive, which follow (1, 3, 4 and Table V):

TABLE V.—Student's method applied to wheat yields in potato-wheat-clover rotation at Wooster, Ohio

Year	Actual yields		Difference in yields No. 15 over No. 32	Devi-ation from 1.49 (D)	D ²	Gains effected		Differ-ence in gains No. 32 over No. 15	Devi-ation from 2.16 (D)	D ²
	Plot No. 15 ^a	Plot No. 32 ^b				Plot No. 32	Plot No. 15			
	Bush.	Bush.	Bush.			Bush.	Bush.	Bush.		
1895.....		(c)								
1896.....		(c)								
1897.....	41.00	39.17	4.83	3.34	11.16	6.45	6.56	-0.11	2.27	5.15
1898.....	37.42	32.25	5.17	3.68	13.54	8.52	13.73	-5.21	7.37	54.32
1899.....	35.67	34.58	1.09	.40	.16	10.69	7.87	2.82	.66	.44
1900.....	48.00	44.91	3.09	1.60	2.56	13.91	5.59	8.32	6.16	37.95
1901.....	29.83	28.33	1.50	.01	.00	7.39	6.55	.84	1.32	1.74
1902.....	52.25	49.33	2.92	1.43	2.05	17.78	15.78	2.00	.16	.03
1903.....	38.67	38.17	.50	.99	.98	13.89	9.20	4.69	2.53	6.40
1904.....	27.50	29.21	-1.71	3.20	10.24	7.43	13.43	-6.00	8.16	66.59
1905.....	34.58	30.13	4.45	2.96	8.76	11.46	11.41	.05	2.11	4.45
1906.....	50.71	49.17	1.54	.05	.00	12.49	6.16	6.33	4.17	17.39
1907.....	35.00	40.83	-5.83	7.32	53.58	6.22	.44	5.78	3.62	13.10
1908.....	48.50	49.50	-1.00	2.49	6.20	17.99	12.06	5.93	3.77	14.21
1909.....	44.87	42.75	2.12	.63	.40	8.68	3.32	5.36	3.20	10.24
1910.....	33.54	34.12	-.58	2.07	4.28	9.10	11.72	-2.62	4.78	22.85
1911.....	39.25	36.33	2.92	1.43	2.04	17.94	14.36	3.58	1.42	2.02
1912.....	13.83	11.04	2.79	1.30	1.69	4.82	2.08	2.74	.58	.34
Mean.....	38.35	36.86	1.49	-----	7.35 σ=2.7	10.92	8.77	2.16	-----	16.08 σ=4.01

Z=0.55.
P=±0.9734.
Odds=37 to 1.

Z=0.54.
P=±0.9715.
Odds=34 to 1.

^a Fertilizer treatment consists of 32 pounds of nitrate of soda, 480 pounds of acid phosphate, and 300 pounds of muriate of potash per acre applied for potatoes only.
^b Fertilizer treatment consists of 16 tons of barnyard manure applied for wheat.
^c No yields are reported for plot No. 32 for 1895 and 1896.

When Student's method of interpretation is applied to the actual yields obtained on plots Nos. 15 and 32 certainty is established in terms of odds of 37 to 1 that the complete chemical fertilizer applied for potatoes, on plot No. 15, is more effective in increasing the yields of wheat (the crop following potatoes) than 16 tons of manure

applied directly for wheat, on plot No. 32; and that when the parallelism of the gains is subjected to the same method of interpretation exactly the opposite conclusions are reached, namely, the odds are 34 to 1 that it is the manure and not the chemical-fertilizer treatment which is the more effective.

It is not the aim here to discredit Student's method. Its advantages are fully recognized. Nor is it the intention of the writer to raise at this time any question as to the certainty of significant differences in fertilizer effects, especially in those cases where, largely because of soil heterogeneity, the interpretation, when based on the parallelism of gains, leads to conclusions opposite to those arrived at when the interpretations are based on paired yields. (This latter point presents a distinct problem, since it is well known that the response made by a soil type to a given fertilizer treatment will vary more or less on local areas or spots, depending in a large measure upon the kind of fertilizer and the degree of difference in the natural producing power of the soil.) The writer wishes simply to call attention to the fact that this method of interpreting fertilizer results has its limitations.

The facts are that natural soil productivity is not a constant on all the plots of a fertility experiment and that the application of Student's method to paired yields may not lead to correct interpretations of fertilizer results. When Student's method is applied to yields soil homogeneity is assumed, but when the method is applied to paired gains soil heterogeneity enters into consideration. Thus it seems more correct statistically and more logical (in order to meet the conditions under which many fertilizer experiments are conducted) to apply Student's method, wherever possible, to parallel gains as a better test of the certainty of any significant difference in fertilizer effects.

A study of the principles on which Student's method is based and of the statistical formulas on which its proof rests can hardly lead one to restrict its application to paired yields. Furthermore, in experiments in which the effects of different fertilizer treatments are compared, the interest does not center so much on the yields as on the gains effected, particularly when such gains can be expressed as "deviation from normal," or deviations from control-plot treatment, assuming progressive differences between control plots. Thus from both the practical and economic points of view it is the difference between gains effected, or second differences, which finally determines which of two fertilizer treatments is the more effective and also perhaps the more profitable.

It has long been the practice to measure the comparative values of different fertilizers in terms of margin of profit over the cost of the fertilizers as determined by the market value of the increases obtained. This is highly desirable from the economic or profit point of view. However, there remains to be given alongside these profits, which serve to measure the comparative economic values of any two fertilizers, some mathematical expression of the statistical significance of the effects of one fertilizer treatment when compared with the other. This can be done consistently, and probably with less error as affecting conclusions, when the interpretation of results is based on the parallelism of gains rather than of actual yields.

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CROWN-GALL STUDIES OF RESISTANT STOCKS FOR PRUNUS¹

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INTRODUCTION

Of all nursery troubles in California on the stocks used for the stone fruits, the disease commonly known as crown gall is probably the most prevalent. Observations in the nursery often reveal a high percentage of infection with crown gall, especially in sandy soils, on peach and almond roots, and to a less extent on myrobalan plum and apricot.

Investigation (3)² has shown that the nursery stocks in general use for the stone fruits in California are susceptible to artificial inoculation with the crown-gall organism, *Pseudomonas tumefaciens* Sm. and T. Cannery pits of peach and apricot, rather than pits of the more primitive species, are usually planted for such nursery stock. Inoculations of the peach (*Amygdalus persica*) produce about 80 per cent infection, and the almond (*Amygdalus communis*) is probably as susceptible. Inoculations of the myrobalan plum (*Prunus cerasifera*) result in 75 to 95 per cent of galls. The apricot (*P. armeniaca*) is somewhat more resistant, infections resulting from about 74 per cent of the inoculations.

The general characteristics of the crown-gall disease caused by *Pseudomonas tumefaciens* have been carefully studied (6), but a satisfactory and practical control is yet to be found. The most promising line of attack is in the use of more resistant stocks, species, or varieties of *Prunus* or *Amygdalus* which in experiments or practice have shown themselves substantially free from infection, and which are also suitable for growth.

The genus *Prunus* is rich in species and varieties which might be employed for stock purposes. Little is known concerning their adaptability as roots for the stone fruits or their resistance to crown gall; these questions are under investigation at the present time. This paper gives a preliminary report on the inoculation of a number of species and varieties of *Prunus* and *Amygdalus*.

SOURCE OF MATERIAL

PRUNUS FORMS

The species listed in Tables I and II have been obtained from supposedly reliable sources. Scions and pits of many of the eastern varieites were obtained from the Arnold Arboretum. The United States Department of Agriculture has sent us material from its

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² Reference is made by number (italic) to "Literature cited," p. 971.

foreign and domestic introductions. Several varieties were obtained from reliable nurserymen. The wild varieties of the Pacific States were obtained through the cooperation of forestry supervisors or through personal collection of pits from natural plantings. W. F. Wight furnished pits of some of the eastern varieties.

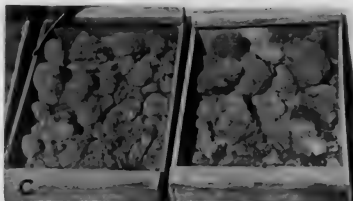
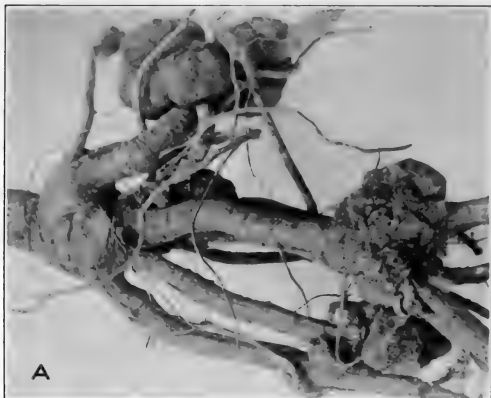
In Tables I, II, and VIII the following letters are used to indicate the difference sources from which the plant material used in this study was obtained:

- AA—Arnold Arboretum, Jamaica Plains, Mass. (Seeds and scions.)
- ESC—Author's collection, Citrus Experiment Station, University of California. (Seeds and scions.)
- ESK—Kansas Agricultural Experiment Station. (Seed from Albert Dickens.)
- ESO—Oregon Agricultural Experiment Station. (Scions from H. P. Barss.)
- EST—Texas Agricultural Experiment Station. (Seed and scions from H. Ness.)
- NB—Luther Burbank Nursery, Santa Rosa, Calif. (Seedlings.)
- NC—Leonard Coats Nursery, Morgan Hill, Calif. (Budded trees.)
- NF—Fancher Creek Nursery (Geo. C. Roeding, manager), Fresno, Calif. (Budded trees.)
- NLC—L. R. Cody Nursery, Saratoga, Calif. (Scions.)
- NP—Theodore Payne Nursery, Los Angeles, Calif. (Seeds.)
- NS—Shenandoah Nursery, Shenandoah, Iowa. (Seedlings and budded trees.)
- NT—Tribble Brothers Nursery, Elk Grove, Calif. (Root cuttings.)
- UCP—C. A. Pampus through Department of Botany, University of California. (Seed.)
- USDA—United States Department of Agriculture, Office of Seed and Plant Introduction. (Seedlings and budded trees.)
- USDAF—United States Department of Agriculture, Forestry Service. (Seeds.)
- USDAH—United States Department of Agriculture, San Antonio Experimental Farms, Texas. (Buds from H. S. Hastings.)
- USDAM—United States Department of Agriculture, Bureau of Plant Industry, Silas C. Mason. (Seed.)
- USDAW—United States Department of Agriculture, Bureau of Plant Industry, W. F. Wight. (Seeds.)

The different species were verified by a careful comparison with scientific descriptions, and in doubtful cases were further authenticated by submitting material to a botanist who had specialized in the genus *Prunus*. The horticultural varieties were studied critically but in many cases could not be positively identified because of their failure to fruit. This is especially true of the varieties of *P. domestica*. (See Table II.) Italian Prune, German Prune, Reine Claude (Green Gage), President, and Bartlett fruited and were apparently correctly named. The other varieties in Table II were not authenticated, but the names were used as labeled by nursery.

THE CROWN-GALL ORGANISM

The crown-gall organism used in 1913 and 1914 in the inoculations on *Prunus* was isolated from the almond. The organism was reisolated in 1914 from *Schinus molle*, and in 1916 and 1918 from two species of *Prunus*. The cultures used were always in a virulent condition, as indicated by the control inoculations, which resulted in 80 to 100 per cent infection. They were never more than two years from isolation. In 1921 a new culture, the last used, was obtained from a natural gall found on *Prunus besseyi*. In all, five different cultures have been used, three of which were from artificial galls produced by the original culture or by cultures reisolated from them.



A.—Galls which developed in two years on a small seedling of *Empodalus daridiana*. Inoculation by mixing fragments of crown galls with soil at time of planting

B.—A pile of crown-gall fragments as they were prepared for use in inoculating soil in these experiments

C.—Boxes showing nature of gall material before being broken up for use in the experiments

EXPERIMENTAL METHODS

For several years inoculation tests have been made each week from May to September (4), using pure cultures of *Pseudomonas tumefaciens* isolated from almond, peach, or plum. The artificial inoculations were made in series of 10 or a multiple of 10 on the current season's growth by punctures with a steel needle. No protection was given the puncture inoculations. In each series a susceptible species was included to test the virulence of the culture. The results were very consistent, in that a resistant species usually showed the formation of but few galls, while a susceptible one showed a large number. This is well illustrated in Table VI, which presents the results of several series of inoculations.

In addition to the method outlined above, pits of such species (Table VII) as could be obtained were planted in soil with fragments of galls taken from diseased orchard trees, usually peach roots (pl. 1). These galls were broken up into small fragments by pounding with a heavy tool. The pits were sown the proper distance apart in a furrow 3 or 4 inches deep, and the gall fragments scattered upon them. The pits in the upper part of each row were not inoculated, being left as a control. The furrow was then filled with soil. In another experiment one-year-old trees were planted in soil, mixed with broken pieces of crown gall.

RESISTANCE IN INOCULATION TESTS

Table I presents a summary of the results obtained from the artificial inoculations on species of *Prunus*. These results agree with those previously published (5). In some cases the number of inoculations was too small to make the results entirely conclusive; these results may be considered as only suggestive of the actual resistance. A resistant species usually shows the characteristic at once, and a large number of inoculations, while making the test more conclusive, generally does not change very materially the actual percentage of galls produced.

The name *Prunus* is used in this paper in its inclusive sense, and the species tested are very diverse. Three species, *Prunus ilicifolia*, *P. integrifolia*, and *P. caroliniana*, are evergreens, and are placed by some authorities in *Laurocerasus*. It will be noted that these showed strong resistance. They can not, however, be successfully budded or grafted with any of the cultivated varieties of the stone fruits. Another group of native subdesert species, *P. andersoni*, *P. fasciculata*, *P. fremonti* (*P. eriogyna*), *P. minutifolia*, and *P. microphylla*, while differing among themselves, have certain common characteristics. They are about equally resistant, yielding about 50 per cent of infections. With the exception of the last two species, they grow in the Pacific and Rocky Mountain States. They are interesting forms, but are probably too small to be valuable as standard stocks. These, together with *P. subcordata*, the Sierra plum, are worthy of experimental testing as dwarf stocks.

The varieties of the species *Amygdalus persica* and *A. communis* thus far tested have shown no marked resistance. The bitter almond is the most promising, but in actual nursery practice this variety often is very susceptible. Other species of *Amygdalus*, as *A. mira*

and *A. tangutica*, appear to be more resistant, but so far only a single introduction has been tested by inoculation, and the inoculations of a larger number of seedlings may change their status in this respect.

TABLE I.—Summary of artificial inoculations on species of *Prunus* and *Amygdalus* by cultures of *Pseudomonas tumefaciens*

Species	Common name	Source ^a	Number of inoculations	Number of galls	Per cent of infection
<i>A. communis</i> L.	Bitter almond	NF	190	56	29.4
<i>A. davidiana</i> Zabel	Davidiana peach (S. P. I. 36664)	USDA	250	130	52.0
<i>A. mira</i> (Koehne)	S. P. I. 34601	USDA	650	120	18.5
<i>A. persica</i> L.	Peach	ESC	211	187	88.6
<i>A. persica</i>	Indian cling peach seedlings	EST	610	396	64.9
<i>A. persica</i>	Peach (S. P. I. 40001)	USDA	250	79	31.6
<i>A. persica</i> potanini Ricker	Peach (S. P. I. 40009)	USDA	200	50	25.0
<i>A. tangutica</i> Korsh	Chinese wild almond (S. P. I. 40010)	USDA	430	25	5.8
<i>P. alleghaniensis</i> Porter	Allegheny plum	AA	250	12	4.8
<i>P. andersoni</i> A. Gray	Desert peach	USDAF	70	30	42.9
<i>P. angustifolia</i> Marsh	Chickasaw plum	ESC	140	37	26.4
<i>P. angustifolia</i> watsoni Waugh	Sand plum	ESK	180	69	38.3
<i>P. americana</i> Marsh	American plum	AA	180	129	71.6
<i>P. armeniaca</i> L.	Apricot (S. P. I. 32834)	USDA	60	5	8.3
<i>P. armeniaca</i>	Blenheim apricot	NF	80	76	95.0
<i>P. armeniaca</i>	Royal apricot	NF	170	140	82.3
<i>P. armeniaca</i>	Royal apricot seedlings	ESC	50	39	78.0
<i>P. armeniaca</i>	Tilton apricot	NF	70	52	74.3
<i>P. armeniaca</i>	Apricot (S. P. I. 17154)	USDA	140	60	42.8
<i>P. armeniaca</i> mandshurica Maxim.		AA	170	39	22.9
<i>P. besseyi</i> Bailey	Bessey cherry	NS	280	29	10.4
<i>P. besseyi</i> Hybrid	Sapa plum	NS	90	54	60.0
<i>P. besseyi</i> Hybrid	Compass plum	NS	50	12	24.0
<i>P. caroliniana</i> Ait.	Cherry-laurel	NP	150	0	0.0
<i>P. cerasifera</i> Ehrh	Myrobalan plum	NF, AA	950	725	76.3
<i>P. cerasifera</i> divaricata Bailey		AA	100	94	94.0
<i>P. cerasifera</i> pissardi Koehne	Purple-leaf plum (seedling)	ESC	50	35	70.0
<i>P. cerasifera</i> Hybrid	Marianna	NS	90	59	65.6
<i>P. dasycarpa</i> Ehrh	Purple apricot	AA	210	94	44.8
<i>P. domestica</i> L.	See Table II				
<i>P. emarginata</i> villosa Sudworth		ESO	40	39	97.5
<i>P. fasciculata</i> A. Gray	Desert almond	ESC	20	4	20.0
<i>P. fremonti</i> S. Wats	Desert apricot	USDAM	90	48	53.3
<i>P. hortulana</i> Bailey	Hortulana plum	AA	130	108	83.1
<i>P. hortulana</i>	Golden Beauty plum	AA	190	51	26.8
<i>P. ilicifolia</i> Walp	Hollyleaf cherry	NF	150	0	0.0
<i>P. insititia</i> L.	Shropshire plum	NF	230	35	15.2
<i>P. insititia</i>	St. Julian plum	NS	110	11	10.0
<i>P. lyoni</i> Sarg	Catalina cherry	NP	80	3	3.7
<i>P. maritima</i> Marsh	Beach plum	AA	140	48	34.3
<i>P. mexicana</i> S. Wats	Big-tree plum	USDAW	280	167	59.6
<i>P. microphylla</i> Hemsl	Mexican almond	UCP	60	29	48.3
<i>P. minutiflora</i> Engelm	Texas almond	USDAH	60	30	50.0
<i>P. munsoniana</i> Wight and Hedr	Wildgoose plum	AA	160	118	73.7
<i>P. mume</i> Sieb. and Zucc	Japanese apricot	USDA	4,950	307	6.0
<i>P. nigra</i> Ait.	Canada plum	AA	80	76	95.0
<i>P. orthosepala</i> Koehne		AA	90	80	88.8
<i>P. pumila</i> L.	Sand cherry	USDAW-AA	450	1	0.2
<i>P. serotina</i> Ehrh	Black cherry	NB	150	24	16.0
<i>P. simoni</i> Carr	Apricot plum	NF	130	130	100.0
<i>P. spinosa</i> L.	Blackthorn plum	NF	80	57	71.2
<i>P. subcordata</i> Benth	Sierra plum	USDAF	15	3	20.0
<i>P. tomentosa</i> Thunb	Nankin cherry	AA	25	22	88.0
<i>P. umbellata</i> Ell	Black sloe (S. P. I. 38974)	USDA	150	1	0.6
<i>P. umbellata</i>	Seedlings (S. P. I. 38974)	ESC	690	173	25.0
<i>P. umbellata</i> injuncunda Sarg		AA	25	22	88.0

^a The explanation of the letters used in Tables I, II, and VIII may be found under "Source of Material."

Prunus armeniaca has not thus far shown any marked resistance in any of the horticultural varieties tested. *P. armeniaca mandshurica*, considered by some a distinct species, is somewhat resistant.

The introduction *P. armeniaca* (S. P. I. 32834) also seems promising, but has not been sufficiently tested.

The varieties of the European plum (*Prunus domestica*) are placed together in Table II. This species contains a great diversity of varieties, some very resistant and others rather susceptible. Six of the seventeen tested show 15 per cent or less of galls developing from artificial inoculations. Is this what may be expected when a number of different varieties of a species are tested by artificial inoculations? *P. insititia*, the damson group, is in many respects very similar to *P. domestica*. It shows a very satisfactory resistance.

TABLE II.—Artificial puncture inoculations on varieties of *Prunus domestica*, arranged in order of resistance

Variety	Source ^a	Number of inoculations	Number of galls	Per cent of galls
Italian Prune.....	NF.....	340	13	3.8
Golden Drop.....	NF.....	160	14	8.7
German Prune.....	NF.....	350	31	8.8
Washington.....	NC.....	60	6	10.0
President.....	NC.....	140	19	13.5
Giant.....	NC.....	60	9	15.0
P. D. stock ^b	NT.....	420	77	18.3
Columbia.....	NC.....	80	20	25.0
Black Damask.....	NLC.....	20	6	30.0
Reine Claude (Green Gage).....	NF.....	160	62	32.5
Sergeant.....	NC.....	130	49	37.7
Red Magnum Bonum (Red Egg).....	NF.....	80	26	38.7
Jefferson.....	NC.....	110	49	44.5
St. Martin.....	NC.....	130	81	62.3
Grand Duke.....	NC.....	60	42	70.0
Bartlett.....	NC.....	60	51	85.0
Sugar.....	NF.....	70	67	95.7

^a The explanation of the letters used in Tables I, II, and VIII may be found under "Source of material."
^b Stock of *Prunus domestica*, used to a limited extent in California. This material was obtained as suckers from orchard trees budded or grafted on this root. The stock can be propagated rather easily by root cuttings.

SOME RESISTANT SPECIES

The species showing marked resistance are listed in Table III, and, with the exception of *Prunus ilicifolia*, *P. caroliniana*, and *P. serotina*, merit further field tests as stocks for the stone fruits. Some of them have been used in a limited way, while others probably have never been given experimental trial as stocks.

TABLE III.—Species of *Prunus* and *Amygdalus* showing less than 16 per cent infection by artificial inoculations with pure cultures of *Pseudomonas tumefaciens*

Species	Common name	Number of inoculations	Number of galls	Per cent infection
<i>P. ilicifolia</i>	Holly-leaf cherry.....	150	0	0.0
<i>P. caroliniana</i>	Cherry-laurel.....	150	0	0.0
<i>P. pumila</i>	Sand cherry.....	450	1	0.2
<i>P. umbellata</i>	Black sloe.....	150	1	0.6
<i>P. domestica</i> ^a	Italian prune.....	340	13	3.8
<i>P. alleghaniensis</i>	Allegheny plum.....	250	12	4.8
<i>A. tangutica</i>	Chinese wild almond.....	430	25	5.8
<i>P. mume</i>	Japanese apricot.....	4,950	307	6.0
<i>P. domestica</i>	Golden Drop plum.....	160	14	8.7
<i>P. domestica</i>	German prune.....	350	31	8.8
<i>P. besseyi</i>	Sand cherry.....	280	29	10.4
<i>P. insititia</i>	St. Julian plum.....	110	11	10.0
<i>P. serotina</i>	Black cherry.....	150	21	14.0
<i>P. insititia</i>	Shropshire plum.....	230	35	15.2

^a Only one of the six varieties listed in Table II as showing 16 per cent or less infection is given here.

Four different seedlings of the sand cherry, *P. pumila*, have been used in the inoculation tests, and it seems to be somewhat more resistant than the variety of *P. besseyi*, known as the Rocky Mountain Dwarf. This stock was purchased from an eastern nursery, and it is not known whether the inoculations were made on seedlings or on stock grown from cuttings from a single source. *P. pumila* grows readily from cuttings planted in the spring, and the same is probably also true of *P. besseyi*. The black sloe (*P. umbellata*) is rather promising. It produces a good-sized tree and does not sucker badly. *Amygdalus tangutica* may be valuable as a dwarf peach and almond stock, though it is said to sucker freely, which is an objection. *P. mume* is the most promising species, and will be considered later. *Amygdalus mira* (the smooth-pit peach) and *A. persica potanini* are promising. They seem to be vigorous growers and should be further tested.

The results are given in Tables I to III can be summarized by arranging the species of *Amygdalus* and *Prunus* in the following groups:

- (1) *Amygdalus*: *mira*, *tangutica*, *persica*, *communis*; 3 to 88 per cent.
- (2) Evergreen species (*Laurocerasus*): *caroliniana*, *ilicifolia*, *lyoni*; 0 to 3 per cent of infection.
- (3) Sand plums: *pumila*, *besseyi*; 0.2 to 10 per cent.
- (4) *Domestica*: 17 varieties tested (Table II); 3 to 95 per cent.
- (5) Damson (*insititia*): St. Julian, Shropshire; 10 to 87 per cent.
- (6) Subdesert species: *andersoni fasciculata*, *fremonti*, *microphylla*, *minutifolia*; 20 to 50 per cent.
- (7) Sloelike plums: *alleghaniensis*, *spinosa*, *umbellata* (S. P. I. 38974 and seedlings of *umbellata* (S. P. I. 38974); 0.6 to 88 per cent.
- (8) Chickasaw plum: *angustifolia*, *angustifolia watsoni*; 24 to 37 per cent.
- (9) Myrobalan plum (*cerasifera*): seedlings, var. *divaricata*, and seedlings of var. *pissardi*; 75 to 90 per cent.
- (10) Apricot (*armeniaca*): Royal, Royal seedlings, Blenheim, Tilton, etc., and var. *mandshurica*; 8.3 to 95 per cent.
- (11) Japanese apricot (*mume*): 46 seedlings (Table V); 1.5 to 13 per cent.

THE BLACK SLOE (*PRUNUS UMBELLATA*)

This species is one of the most promising of the native forms of *Prunus* for use as a rootstock for the stone fruits. It has been used for rootstocks in Florida. In California, under the climatic conditions of Riverside, it makes, the first season, a stocky growth of 3 to 4.5 feet in height. Tests are under way to determine its adaptability for the different varieties of the stone fruits. The results of the artificial inoculations as listed in Table I showed a single tree of *P. umbellata* (S. P. I. 38974) as less susceptible than *P. umbellata injucunda* (*P. mitis* Beadle), but further inoculation tests of the former on more rapidly growing wood are necessary before its crown-gall resistance can be considered as established.

In Table IV are given the tabulated results of inoculation of seedlings of *Prunus umbellata* (S. P. I. 38974). Control inoculations were made on seedlings of Indian Cling peach. Eighty puncture inoculations gave 53 galls or 66 per cent infection, which result agrees very closely with 64.9 per cent given for this form of *P. persica* in Table I.

TABLE IV.—Results of artificial inoculations in 1924, on seedlings of *Prunus umbellata* (S. P. I. 38974)

Number of seedlings	Age (years)	Number of inoculations	Number of galls	Per cent of infection
1	3	90	0	0.0
1	3	90	2	2.2
1	3	110	3	2.7
1	2	60	5	8.3
1	2	60	5	8.3
1	2	60	5	8.3
1	3	70	9	12.8
1	3	80	23	28.7
1	3	80	28	35.0
1	3	110	44	40.0
1	3	100	49	49.0
11	-----	910	173	* 19.0

* Average.

The seedlings whose inoculations are listed in Table IV were all growing vigorously, and were in favorable condition to give a high per cent of crown-gall infection. The seedlings showed considerable variation in their susceptibility, but in most cases they apparently were more susceptible than the parent tree. More than half of them (7) showed less than 16 per cent infection, and could well be listed with the resistant forms as given in Table III. The results suggest, at least, that with proper selection a resistant form of this species could be developed. An asexual method of propagation would of necessity have to be employed. From a limited test, the root-cutting method seems promising.

THE JAPANESE APRICOT (*PRUNUS MUME*)

The species most thoroughly tested is the Japanese apricot, *Prunus mume*. The results of the tests are summarized in Table V. Two of the introductions, S. P. I. 28685 and 43558, were budded on other stock. The other three introductions were seedlings grown from pits imported through the Bureau of Plant Industry of the United States Department of Agriculture. The pits from S. P. I. 46694 and 47950 were grown on the California Citrus Experiment Station grounds. S. P. I. 45523 consisted of 25 small trees obtained from the United States Department of Agriculture. It will be observed (Table V) that there is considerable variation in the percentage of galls that developed. S. P. I. 46694 is exceedingly low in this ratio, while S. P. I. 45523 is relatively high. The other three are practically alike. This variation in susceptibility can also be noted in the individual trees of introduction S. P. I. 45523. The trees of the other introductions are all practically alike in susceptibility, being very resistant.

The galls that develop on the resistant trees of *Prunus mume* are small in size, sometimes mere pointlike growths that have formed on the healing tissue at the margin of the wound. These are usually less than one-sixteenth inch in height, with about the same diameter at the base. As to the shape, they are pointed rather than sub-globose. It is very probable that most of these smaller pointlike galls will not further increase in size. Resistance, while suggested

by the small size and characteristic shape of the galls, is demonstrated by the small number that develop. These characteristics of gall development have been observed (3) in other resistant species, especially in the case of *P. domestica* (Italian prune and German prune) (pl. 3, B to E).

TABLE V.—Summary, by years of inoculations, on different introductions of *Prunus mume*

Year	S. P. I. 28685 ^a (1)	S. P. I. 43558 ^a (1)	S. P. I. 45523 ^a (21)	S. P. I. 46694 ^a (12)	S. P. I. 47950 ^a (11)
1916.....	^b 30 0				
1917.....	120 10				
1918.....	120 16				
1919.....	60 1	^b 40 8	^b 70 13		
1920.....	20 0	20 0		^b 150 0	^b 600 34
1921.....	70 5	70 8	420 54	620 20	260 20
1922.....	80 8	50 0	840 122	810 4	530 2
Total.....	500 40	180 16	1,330 189	1,580 24	1,390 56
Per cent of galls.....	8.0	8.9	14.2	1.5	4.0

^a The catalogue number assigned by the Office of Seed and Plant Introduction, Bureau of Plant Industry, United States Department of Agriculture. The number in parentheses indicates the number of different seedlings inoculated.

^b The first number indicates the number of inoculations, the second the number of galls that developed.

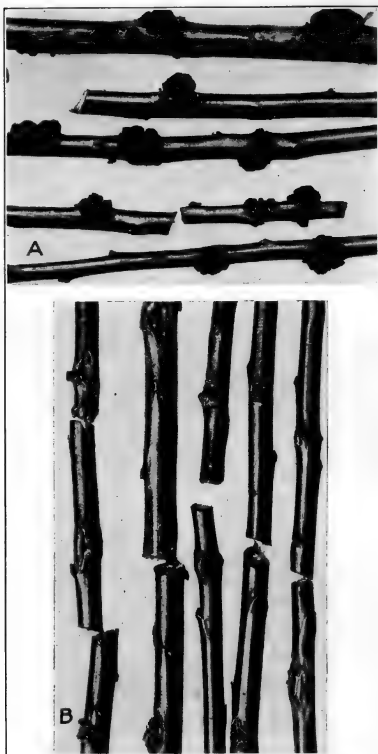
On the more susceptible introductions of *Prunus mume* the galls are much larger and subglobose in shape. They are similar to typical crown galls that develop on other hosts from artificial inoculations.

TABLE VI.—Number of galls which developed in each series of 10 punctures in inoculated species, resistant and susceptible, of *Prunus* and *Amygdalus*

Species	Common name	Number of galls developing from each 10 punctures ^a
<i>P. domestica</i>	Italian prune.....	0, 1, 0, 0, 0, 0, 0, 4, 0, 2, 0, 0, 2, 0, 0, 3, 0, 0, 0, 0, 1, 0
<i>P. domestica</i>	German prune.....	0, 0, 0, 0, 6, 0, 0, 6, 0, 2, 0, 0, 0, 0, 0, 2, 0, 0, 0, 0, 0
<i>P. besseyi</i>	Sand cherry (Rocky Mountain Dwarf).....	1, 0, 2, 2, 0, 0, 4, 0, 0, 0, 0, 0, 0, 0
<i>P. mume</i>	S. P. I. 46694 (Japanese apricot.).....	0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 6, 0, 2, 2, 0, 2, 0, 0, 0, 0, 1, 0, 0, 1
<i>P. mume</i>	S. P. I. 47950 (Japanese apricot.).....	3, 0, 3, 8, 7, 6, 0, 5, 3, 5, 1, 0, 0, 1, 4, 4, 1, 0, 0, 2, 1, 0, 0, 1, 0, 1, 0, 0
<i>P. angustifolia watsoni</i>	10, 0, 0, 5, 0, 0, 6, 6, 7, 4, 3, 4
<i>P. alleghaniensis</i>	Allegheny plum.....	0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 3, 0, 5
<i>A. tangutica</i>	Chinese wild almond.....	0, 3, 1, 0, 1, 0, 0, 0, 0, 1, 1, 4, 0, 1, 0, 6, 6, 1, 0, 0, 0, 0, 1, 0, 0
<i>P. cerasifera</i>	Myrobalan plum.....	10, 8, 9, 8, 5, 8, 0, 4, 2, 4, 10, 2
<i>P. cerasifera divaricata</i>	10, 10, 9, 7, 10, 8, 10, 10, 10, 10
<i>A. persica</i>	Elberta peach.....	9, 10, 10, 10, 9, 0, 10, 10, 7, 8, 0, 10, 10, 10
<i>A. communis</i>	Bitter almond.....	8, 10, 0, 2, 0, 0, 0, 0, 0, 0, 8, 7, 0, 4, 0
<i>A. davidiana</i>	Davidiana peach.....	10, 10, 10, 9, 9, 10, 10, 8, 0, 2, 10, 8, 0

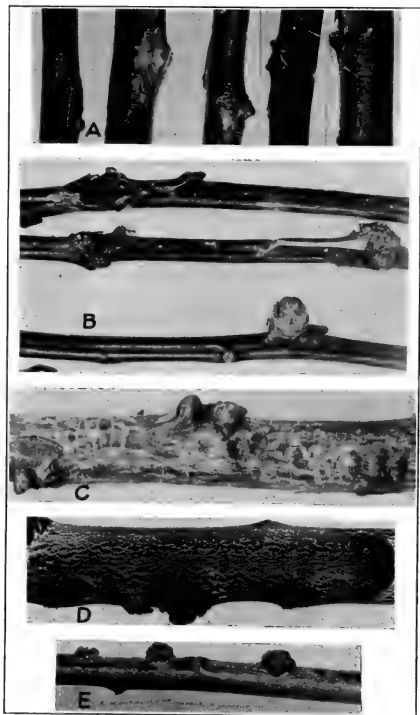
^a Data do not include all the inoculations that were made.

Riker (2), experimenting with some inoculations on tomatoes, found that the size of the original puncture determined the size of the gall. The difference in size of the original puncture could hardly explain the relatively small size of the galls that developed on the resistant trees of *Prunus mume* as compared with the larger ones that appeared on the susceptible trees, since in all these inoculations a steel needle of the same size was used. The resulting wounds on



A.—Artificial inoculation on a susceptible variety of *Prunus mume* (S. P. I. 45523). The galls are typical crown-gall hypertrophies. July to December, 1922

B.—Artificial inoculations on a resistant variety of *Prunus mume* (S. P. I. 47950). Small pointlike hypertrophies are at the margins of the healing tissue. These hypertrophies, for the most part, do not increase in size. They are thought to be characteristic of resistance in *Prunus*. July to December, 1922



A.—Artificial hypertrophies on a resistant variety of *Prunus mume* (S. P. I. 47950). Enlarged about twice, to show small incipient galls. The puncture wounds have healed, but near their margins are these small pointlike growths. August to December, 1922.

B.—Artificial galls on *P. armeniaca mandshurica*, to show form characteristic of a somewhat resistant variety of *Prunus*. The hypertrophies are developed at the margins and in one case at nearly right angles to surface of host. $\times 2$. July to December, 1917.

C.—Small hypertrophies on *Prunus armeniaca mandshurica*, to show small pointlike growths on margins of healing tissue. $\times 2$. June, 1916, to March, 1917.

D.—Small galls in *Prunus domestica* (Italian prune), to show nature of galls as they often develop. For illustrations of larger galls see (5). July to December, 1913.

E.—Small galls on *Prunus domestica* (German prune). While these are typical galls in shape, their size is small. May to December, 1915.

the resistant trees, as indicated by the scar of the healed injuries, were of a greater size than the galls that developed (pl. 2, B, and pl. 3, A). On the susceptible trees the galls for the most part correspond in size with the scar of the healed inoculation (pl. 2, A).

The formation of galls on this host can not always be correlated with growth activity. Negative results often take place on rapidly growing shoots.

SOIL INOCULATIONS BY GALL FRAGMENTS

The resistance of available nursery seedlings was tested in the manner already indicated, by inoculating the soil with fragments of galls at the time of planting. This method has been used by various investigators (?), and gave satisfactory results even before the true cause of the disease was known. It was employed as giving possibly more normal conditions of infection. It would seem to be at least a more natural method than puncture inoculation in the twigs. Small, one-year-old trees were also inoculated by gall fragments in the same way as were the germinating pits. In most instances a much smaller percentage of galls was produced on the small trees by this method than by artificial inoculation in the twigs of the same species. Probably some other factors may have been responsible for this difference. The inoculum must be rather variable in its pathogenicity when broken fragments of galls are used, although care was taken to collect fresh galls and use them very soon after they were broken up.

TABLE VII.—*Results after two years' growth from germinating pits planted with minced peach galls*

Stocks	Soil inoculated		Soil not inoculated	
	Number of trees	Number with galls	Number of trees	Number with galls
<i>P. andersoni</i>	17	1	6	1
Peach.....	30	28	32	1
Peach ^a	29	26	42	0
Peach-almond hybrid(?).....	52	45	34	0
Almond (hard-shell).....	57	36	42	1
Almond (bitter).....	101	82	78	4
Almond (sweet).....	98	79	42	1
Apricot (Royal).....	46	22	42	0
Apricot ^a	42	16	25	2
<i>P. fremonti</i>	47	18	-----	-----
Almond (sweet).....	71	49	-----	-----
Peach.....	47	25	-----	-----
Almond.....	35	25	-----	-----
<i>A. davidiana</i>	19	11	-----	-----
<i>P. mume</i> ^b	16	0	-----	-----
Almond ^c	16	10	-----	-----

^a Treated with Bordeaux paste.

^b Trees examined, but not dug up.

^c As control on *P. mume*.

USES OF RESISTANT STOCK

A strongly resistant stock would have another use, in addition to that of propagating new trees. It could be used for inarching in orchards where the trees are already diseased with crown gall but not in too decadent condition. The inarching of small trees can

readily be performed without any great technical skill. A limited amount of experimental work in inarching *Prunus* into diseased apricot trees on peach stock has given very satisfactory results.

TABLE VIII.—Results after two years' growth in soil inoculated with minced galls at the time of planting 1-year-old trees, 6 to 8 inches high

Stock	Common name	Source ^a	Number of trees	Number with galls
<i>P. cerasifera</i>	Myrobalan.....	NS.....	139	4
<i>P. cerasifera</i> (hybrid).....	Marianna.....	NS.....	155	7
<i>P. insititia</i>	St. Julian plum.....	NS.....	48	2
<i>A. davidiana</i>	Davidiana peach.....	USDA.....	41	35
<i>P. americana</i>	American plum.....	AA.....	56	2
<i>P. munsoniana</i>	Wildgoose plum.....	AA.....	18	0
<i>P. besseyi</i>	Sand cherry.....	NS.....	49	2
<i>P. mume</i>	Japanese apricot.....	USDA.....	16	0
<i>P. domestica</i>	P. D. stock ^b	NT.....	27	0
<i>P. americana</i>	Sprouts from old tree ^c	17	0

^a The explanations of the letters used in Tables I, II, and VIII may be found under "Sources of material."

^b *Prunus domestica*, a rootstock secured from sucker in a commercial orchard, and used to a limited extent in California.

^c The sprouts of *P. americana* were secured from a tree (now dead) in the Botanical Garden, University of California.

GERMICIDES IN THE NURSERY

In the tests reported in Table VII certain of the germinating pits of apricot and peach were covered with a Bordeaux paste before planting. When this had become dried the pits were planted with and without minced galls. After two years no difference could be found between these trees and those growing in adjacent rows not treated with Bordeaux paste but inoculated with gall fragments.

Table IX is a summary of tests made by using different germicides on peach roots to protect them from infection with the gall organism when inoculated with fragments of peach galls. The peach roots were two years old, and none of them showed any evidence of crown gall when planted.

Slight injuries were made on the roots by light scraping before treatment with germicide. The gall fragments were mixed with the soil at the time of planting. The results indicate that under the conditions of the experiment the germicides were not very effective in preventing the development of crown gall, although in all tests but one there was a reduction in the percentage of galled trees. The conditions in all cases were much more favorable for infection than under usual orchard practice, in that there was abundant inoculum and injuries favorable for infection. All the germicides were more or less injurious to the roots, as was indicated by the number of trees dying as compared with the controls. The Bordeaux paste was no more efficient than the 12-15-50 Bordeaux mixture. These results are much less promising than those of Melhus and Manley (1), who, by using Bordeaux paste on bench-grafted apple roots, obtained very good results. The large amount of inoculum present in these tests of the writer may possibly explain the difference in results, and apples are possibly less susceptible than peaches

TABLE IX.—Results from the treatment of 2-year-old peach roots with disinfecting chemicals, after which they were planted in soil infected with freshly broken-up crown galls and then grown for two years

Treatment given	Number of trees dying	Number of trees with galls	Number of trees free from galls	Percentage of trees with galls
Bordeaux paste 3-6-4.....	14	23	14	62
Bordeaux mixture 4-6-50.....	6	20	4	83
Bordeaux mixture 12-15-50.....	12	7	11	38
Ortho lime-sulphur (concentrated).....	5		All died.	
Ortho 1-10.....	2	14	9	60
Ortho 1-20.....	2	7	11	38
Ferrous sulphate (Bordeaux paste)*.....	7	3	18	14
Check.....	0	40	10	80

* This was made by the formula of J. W. Toumey, (7, p. 26): 1 part ferrous sulphate, 2 parts copper sulphate, and 3 parts quicklime.

SUMMARY

Forty different species (often several varieties per species) of *Prunus* and *Amygdalus* have been tested by making artificial inoculations with the crown-gall organism, *Pseudomonas tumefaciens*. There are apparently great differences in susceptibility among these species and often among varieties of the same species. This is especially true in such diversified species as *P. domestica*.

Resistance, from the data given sometimes seem to be a specific and at other times a varietal characteristic. In *Prunus domestica* some of the varieties tested have shown strong resistance, giving infection from 15 per cent or less of the inoculations. In the wild species *P. pumila*, *P. besseyi*, *P. mume*, *P. umbellata*, and *P. alleghaniensis*, the species as a whole is apparently strongly resistant. Further tests may, however, demonstrate that this resistance is a varietal characteristic.

The inoculation by punctures is a test more severe than would be found under natural conditions. The species in Table III probably would be completely resistant under nursery conditions. Possibly those showing less than 25 per cent of infection might also be classed as practically resistant.

Species of *Amygdalus* showed a high percentage of infection when the inoculations were made with gall fragments at the time of planting the pits. The one-year-old trees of various species inoculated by placing gall fragments around the roots showed a lower percentage of infection than those trees inoculated through punctures on the twigs.

Seven years of experimental inoculation on *Prunus mume* has demonstrated its high resistance. From 4,950 inoculations only 307 galls developed, or about 6 per cent of infection. Some of the introductions of this species are more susceptible than others; they show about 13 per cent of infection, which is comparatively low.

Preliminary inoculation tests on seedlings of *Prunus umbellata* indicate a considerable variation as regards susceptibility to crown gall (Table IV). Seven out of eleven of them gave less than 15 per cent infection. The results suggest the possibility of finding a strongly resistant strain of this species.

Amygdalus mira, the smooth-pit peach of China, has shown a very satisfactory resistance. Seedlings of this species are now being tested. *Amygdalus tangutica* and *A. persica potanini* are promising. Seedling peaches from commercial pits and standard commercial varieties are much more susceptible.

The Italian prune and other varieties of *P. domestica* (Table II) have shown resistance, and seedlings of these should be grown to test the inheritance of resistance.

Prunus armeniaca (S. P. I. 32834) and *P. armeniaca mandshurica* seem to show a higher resistance than the commercial varieties of apricot or their seedlings that have thus far been tested.

No practical method for using germicides in treating germinating pits or roots of the peach can as yet be recommended.

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GROWTH-EQUATION CONSTANTS IN CROP STUDIES ¹

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INTRODUCTION

The writers undertook, during the season of 1921, to obtain some experimental data as to the value of the sunflower crop for silage. There was at the time an increasing interest on the part of Illinois farmers in the sunflower as an alternative crop to corn for use as a silage crop. The investigation² was planned primarily for practical ends, and the proper stage of maturity for harvest seemed to be one of the questions on which information was needed.

The Mammoth Russian variety of sunflower was used, and the date of planting was May 18, 1921. The crop was grown at Urbana on a 10-acre field 80 rods long. The crop in three parts of the field was harvested for silage at three stages of maturity—87, 107, and 126 days after planting. The resulting silage was used in digestion and feeding trials in comparison with corn silage for milk production. This represented the limit of the facilities available for harvest and utilization of the crop as silage.

Further data as to the progressive changes in the crop with advance in maturity were obtained by quantitatively sampling the growing crop in the field at eight periods—65, 75, 86, 96, 106, 117, 127, and 138 days after planting. Four rows near the center of the field were reserved for this purpose. The plants for the field samples were selected in a systematic manner. The first plant for the sample at any period was taken at a preselected number from one end of the row. With this plant as a starting point, every hundredth plant was taken at the first sampling period, every ninety-ninth plant at the second period, and so on to every ninety-third plant at the eighth sampling period. The number in the row of the first plant selected for any sample was varied at each sampling period so as to insure some distance between the plants entering into the various samples, and thus avoid, in the samples, the effect of thinning by removal of plants. The plants selected at each period were cut 6 inches above ground level and the combined lot of 58 to 60 plants was at once weighed and subsampled for analysis. Separate determinations were made on stalk and seed, commencing 86 days after planting. Analysis included the analysis customarily made of feed: Dry matter, crude protein, crude fat, crude fiber, nitrogen-free extract, and ash. The

¹ Received for publication Apr. 22, 1925—issued January, 1926.

² More complete data than here given have been published in the following bulletins:

GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407-455, illus. 1925.

NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. FEEDING VALUE FOR DAIRY COWS; COMPOSITION AND DIGESTIBILITY WHEN ENSILED AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 253: 185-225, illus. 1924.

ash was also analyzed for aluminum, calcium, iron, magnesium, phosphorus, sodium, and sulphur. Computations have been made to acre yields on the basis of the number of plants per acre, 14,662. (The rows were 3.38 feet apart, and the plants averaged 10.55 inches apart in the row.)

While the investigation was not planned as a physiological study of plant growth, nevertheless the data accumulated shows something of the growth changes occurring in the sunflower crop. The curves given by the eight points of determination show, in the case of many of the crop constituents as analyzed, the general reverse curve characteristic of growth in annual plants and animals. Following the lead of Robertson,³ particularly, the writers have utilized the general form of equation representing the course of an autocatalyzed monomolecular reaction in deriving a mathematical expression of the data from a growth standpoint. The results have seemed to the writers, rather suggestive as to the possibility of the constants of such growth equations serving a very useful purpose in crop studies. If the constants have the significance attached to them by Robertson, they should be of value in supplementing the data of final crop yield as customarily used in variety tests, etc. At least certain very striking differences appear in the constants as between species such as the sunflower and corn, as indicated by the data at hand. It is with the thought of somewhat emphasizing the differences thus shown that the present paper is offered.

THE AUTOCATALYTIC GROWTH CURVE

A brief review of Robertson's⁴ presentation of the equations expressing the course of an autocatalytic monomolecular reaction will serve to bring out the background as to the significance to be attached to the constants of this sort of growth equation. Representing the amount of material at the start of such a reaction by A , and the amount transformed at any later time by x , then the amount of the original material left at any time is $A - x$. The velocity of the reaction is accelerated (catalyzed) by a product of the reaction present in amount proportional to x . The velocity is also proportional to the amount of original substance left, that is, $A - x$. Hence the velocity at any moment is proportional to $x(A - x)$. Designating time by t , the velocity is expressed as

$$\frac{dx}{dt} = kx(A - x) \quad (1)$$

in which k is a specific constant.

It is obvious from (1) that the velocity will increase to a maximum with time until $x = A - x = \frac{1}{2}A$, after which time the velocity will continually decrease. Equation (1), when integrated, gives the relation

$$\log \frac{x}{A - x} = K(t - t_1) \quad (2)$$

in which $K = kA$, and $t_1 = t$ when $x = A - x$. Time is thus given a negative value preceding the point at which A is one-half transformed, and a positive value thereafter.

³ ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

⁴ ROBERTSON, T. B. Op. cit.

Equation (2) is in form for application to observed data. The approximate application of the equation to growth data is comparatively simple. Figure 1 shows the general form of the curve. It has a lower asymptote of 0 and an upper asymptote of the value of A . If the growth observations are plotted as ordinates against time as abscissas, a reasonable value can be chosen by inspection as representing the upper asymptote, and this value is taken as A . A line is then drawn at $\frac{1}{2}A$ and the point where the growth curve seems to cut this line is taken as t_1 . Time may be taken from any point of origin and reckoned in any unit. In the present data the date of planting has been used as a convenient origin, and the day as a natural and convenient unit of time. For direct comparison between constants of different growth curves it is of course necessary that time be reckoned in the same unit. Having chosen values for

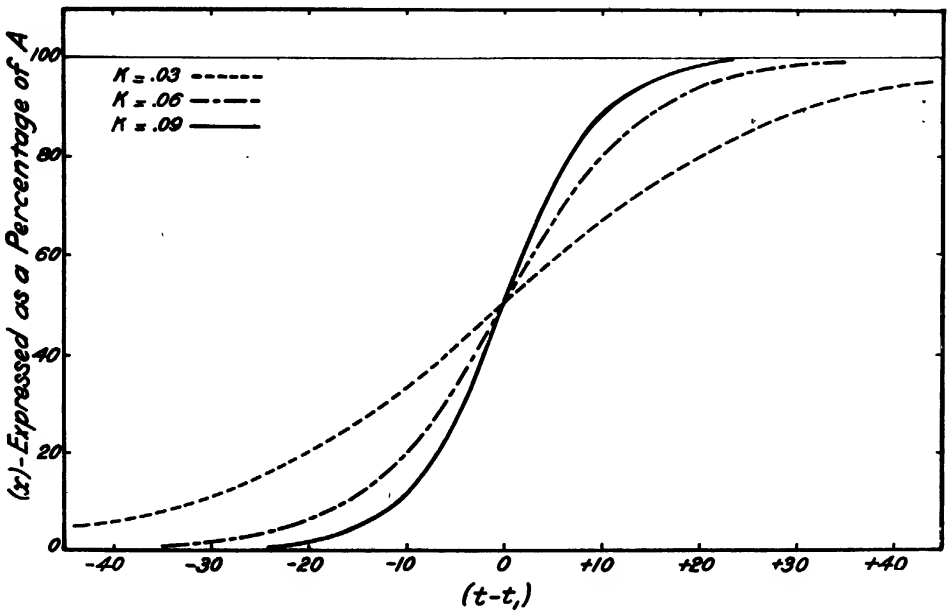


FIG. 1.—Showing form of the curve to the equation $\log \frac{x}{A-x} = K(t-t_1)$ for 3 values of K , 0.03, 0.06, and 0.09. (Note that a higher value of K means a shorter “grand period” of growth. The values of K in this paper lie within the range 0.03 to 0.09.)

A and t_1 the value of K required to satisfy each observation may be computed, and the average of the several values thus obtained is used as the value of K . Robertson’s Table LX⁵ facilitates the computations. The curve is then drawn, and if necessary slightly different values for the constants are chosen. The curve may be changed bodily to right or left by changing t_1 . Its general slope may be increased or decreased by changing K . A better fit of the theoretical curves to the observed data may be obtained by correcting the constants as thus approximated by the method of least squares. For the present purpose the writers have considered this refinement as unnecessary.

The question, from the present standpoint, is, what significance may be attached to the constants of the equation when applied to the observational growth data. According to Robertson’s view, A ,

or the final resultant of the growth processes, is to a considerable degree subject to modification by environmental conditions. On the other hand, k of equation (1) is a specific constant and represents certain inherent qualities of growth of the organism. In equation (2) $K = kA$, and hence after application to the observational data k of equation (1) may be estimated from A and K of equation (2) as $k = K/A$. According to this view K/A affords an index of genetic significance as representing the inherent rate or velocity of growth characteristic of the organism. Thus Robertson⁶ finds that British male infants under the dissimilar environments of England and Australia have dissimilar values of A , namely 318.0 and 341.5, respectively, but similar values of K/A , namely, 0.000399 and 0.000398, respectively. The inference is that like heredity leads to like values of K/A , even though the environment is such as to lead to unlike values of A in the growth equations. If the constant has this genetic significance it affords a highly valuable measure of growth and inherent growth limitations.

In agricultural practice we are concerned with the final growth attained by the crop, and generally this is identical with A of the equation. In some cases, as will appear later, it is necessary to assume a higher value for A than that represented by the crop yield. It is apparent, therefore, that so far as affected by the value of A , the constant K/A varies in an inverse ratio to the crop yield. It will be seen in Figure 1 that a high value of K means a short-growing period, and quite naturally a short-growing period tends to be associated with a low-crop yield. Therefore the effect of K tends to give also an inverse ratio of the constant K/A to yield.

Conversely, A/K will tend to vary directly with crop yield, and consequently the constant in this form is somewhat better adapted for use in comparisons. Where K/A is a specific constant representing inherent growth *velocity* (that is, the rapidity with which full growth is accomplished, not the rate of absolute growth increase), A/K is a specific constant representing inherent final growth *capacity*. A/K is to be regarded, of course, simply as the reciprocal of k of equation (1). Its value as an index of growth capacity or crop yield depends upon the association between the length of time that the crop or crop constituent under consideration continues to grow and the final extent of growth. Experience indicates that length of growing period and extent of growth or crop yield are directly associated. On this reasoning the constant A/K should represent the inherent capacity for crop yield as between genetically different varieties or species under optimum or comparable conditions of environment. How well it may actually serve such a scientifically important and practically useful purpose remains to be demonstrated by critical experiment.

The data of the present paper pertain to the sunflower crop and the corn crop, the data for corn being derived from the work of Jones and Huston.⁷ The data pertain not to individual plants directly but to acre populations collectively. In the graphic presentation the same scale is used for time in days throughout, but the scale

⁶ ROBERTSON, T. B. Op. cit. p. 40.

⁷ JONES, W. J. and HUSTON, H. A. COMPOSITION OF MAIZE AT VARIOUS STAGES OF ITS GROWTH. Ind. Agr. Exp. Sta. Bul. 175: 599-630, illus. 1914.

for growth in pounds is varied, being expressed uniformly as a percentage of the theoretical total or final growth. The sunflower equations may be converted to the average individual plant (upwards of 6 inches above ground) by dividing A by 14,662. The corn equations may be converted to the average individual plant (above ground) by dividing A by 10,000. Just what effect this difference in density of the population may have caused in the constants of the sunflower and corn equations the writers have no means of knowing precisely. Theoretically, A/K should not be affected by this or other environmental factors. At the present stage, of course, the whole theoretical matter may be regarded as no more than a working hypothesis.

CRUDE FIBER AND ALUMINUM IN THE SUNFLOWER

Crude fiber is a constituent of the plant customarily determined in feed analyses. It consists principally of the cellulose tissues of the plant. This constituent corresponds in a way to the skeleton of an

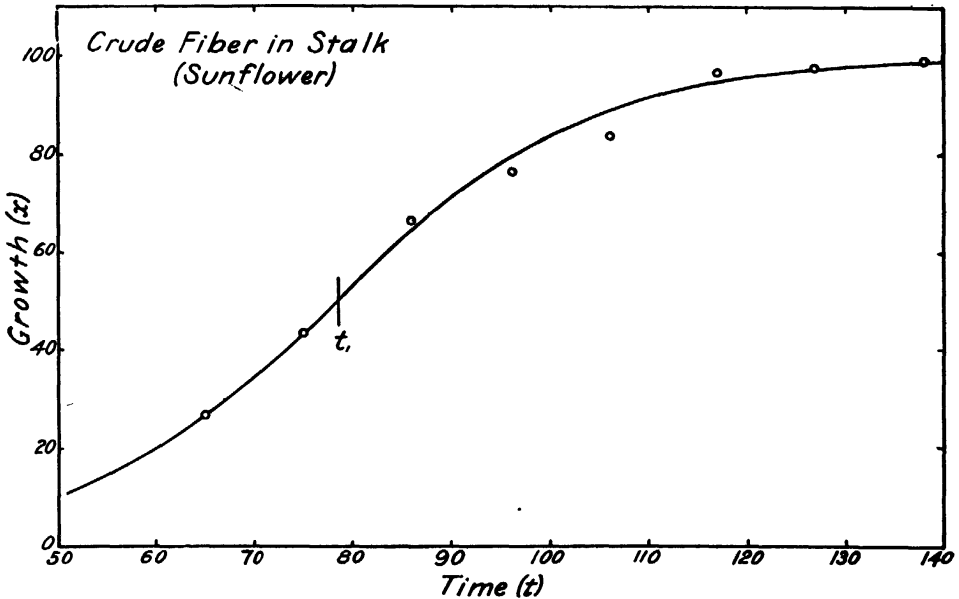


FIG. 2.—Growth of crude fiber in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 2,700 pounds. Equation to curve:

$$\log \frac{x}{2700-x} = 0.033 (t-78.5)$$

animal, and affords a good measure of growth in size or volume of the plant. The observations and fitted curve for the crude fiber in the stalk⁸ are shown in Figure 2. The curve gives a fair fit to the observations, although a considerably better fit is obtained by a logical modification, as shown in Figure 4. The curve is presented in the form given in Figure 2 partly for the sake of comparison with Figure 3, which pertains to aluminum.

The observations and fitted curve for aluminum are shown in Figure 3. Considerable irregularity is manifest in the aluminum

⁸ The seeds were separated carefully by hand, and the stalk includes the remainder of the head and plant as cut 6 inches above ground. In the case of the corn the ears (that is, cob and kernels together) were separated, and the stalk includes the remainder of the ear (husk and shank) and plant as cut at ground level.

data. This irregularity may be partly attributable to difficulty of chemical determination.

Crude fiber and aluminum are unique in that they are the only constituents of the stalk, as determined, that do not undergo more or less marked depletion in the stalk coincident with the development of the seed. This suggests that the two are in some way associated. It is found from the equations given that K is the same in both cases, but that t_1 falls 18.5 days later in the aluminum curve than it does in the crude-fiber curve. If the aluminum curve is shifted 18.5 days to the left, and superimposed on the crude fiber curve, the two curves coincide. Aluminum is thus directly proportional to the crude fiber of 18.5 days preceding. If aluminum is an essential element in the building up of the crude-fiber tissues, its accumulation should occur

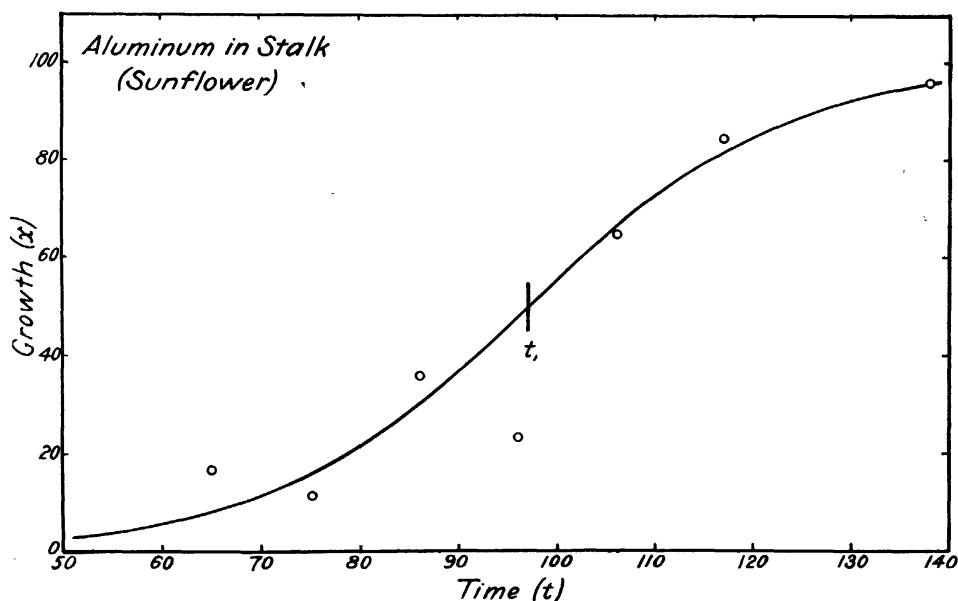


FIG. 3.—Growth of aluminum in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 7.5 pounds. One observation at $t=127$ and $x=15$ (=10.86 pounds per acre) is not included in the graph and was ignored in fitting the curve. Equation to curve:

$$\log \frac{x}{7.5-x} = 0.033 (t-97)$$

simultaneously with or precede that of the crude fiber. On the other hand, if the aluminum represents a nonessential constituent held mechanically by the crude fiber structures of the plant, it might be expected to accumulate in proportion to the crude fiber, but at a later time. The pronounced lag in the appearance of aluminum favors the view that it is associated with the crude fiber as a mere mechanical accumulation, rather than as an essential element of the plant tissues. This illustrates one use of the growth-equation constants, and brings out a relation which might otherwise easily escape detection.

CRUDE FIBER IN STALK OF SUNFLOWER AND CORN

The vegetative and reproductive stages of growth of annual plants represent two distinct aspects of the physiology of growth of the plant as a whole. The present data pertaining to the stalk principally

represent the vegetative phase, but they also include certain portions of the plant directly concerned with the reproductive phase. It is reasonable, therefore, to treat the data as representing two cycles of growth. Such treatment is suggested also by the fact that growth in animals in many cases occurs in two or three distinct cycles.

In Figure 4, the observational data for crude fiber in the stalk of the sunflower are repeated. Two curves of growth (designated x' and x'') are given, together with the sum of these two as representing the observed data. It will be observed at once from Figure 4 that the theoretical curve as thus derived agrees better with the observations than does the single-cycle curve of Figure 2. The root mean-square error for the single-cycle curve is 66 pounds, and for the double-cycle curve 40 pounds.

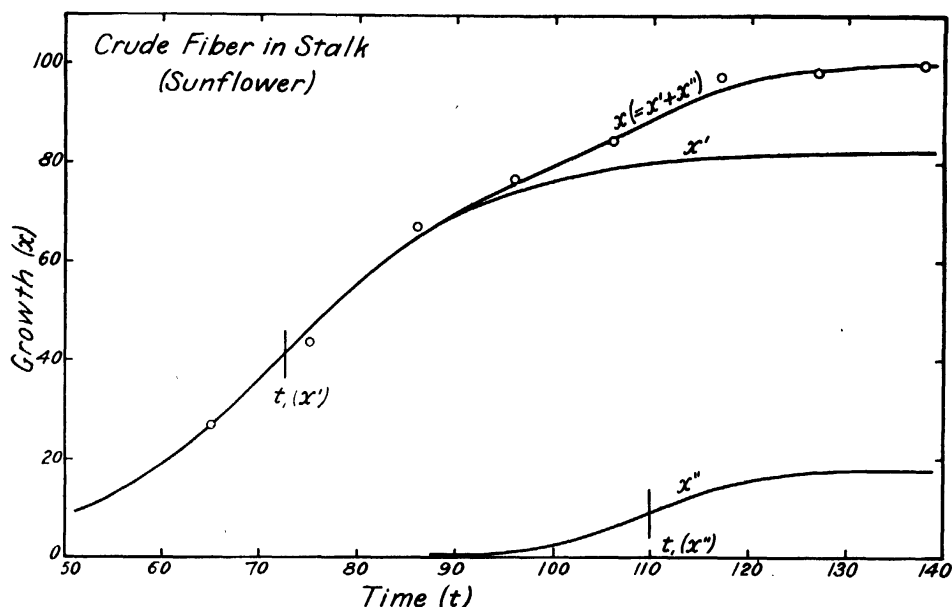


FIG. 4.—Growth of crude fiber in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 2,680 pounds. Observations same as Figure 2, treated as a two-cycle phenomenon. Equations to curves:

$$\log \frac{x'}{2200-x'} = 0.042 (t-72.5)$$

$$\log \frac{x''}{480-x''} = 0.082 (t-110)$$

Reed and Holland⁹ studied the growth of the sunflower plant in height (cm.) and from their data derived the equation

$$\log \frac{x}{254.5-x} = .042 (t-34.2)$$

time being counted in days from a date following planting not definitely stated. K of their equation agrees exactly with K for the first cycle equation of Figure 4, a value considerably different than K of the single-cycle curve of Figure 2. Since growth in height of the plant is not accelerated by seed development, the relation between the K 's of the three equations may be taken as further evidence that the crude-fiber data represent a two-cycle growth process.

⁹ REED, H. S., and HOLLAND, R. H. THE GROWTH RATE OF AN ANNUAL PLANT HELIANTHUS. Proc. Nat. Acad. Sci. 5: 135-144, illus. 1919.

The data for crude fiber in the stalk of corn are presented in Figure 5. Mere inspection of the plotted observations is sufficient to show that they do not conform to the form of curve exhibited by the general equation as illustrated in Figure 1. Treatment of the data, however, as a two-cycle matter yields a theoretical curve that conforms beautifully to the observations. Just how well it may satisfy the reason is perhaps another matter. It involves the assumption that the second growth cycle is at its maximum velocity at the time of the last observation. This assumption, it will be apparent, attaches great weight to the accuracy of the last observational value as representing the course of growth, and should be supported by other experimental work before full acceptance.

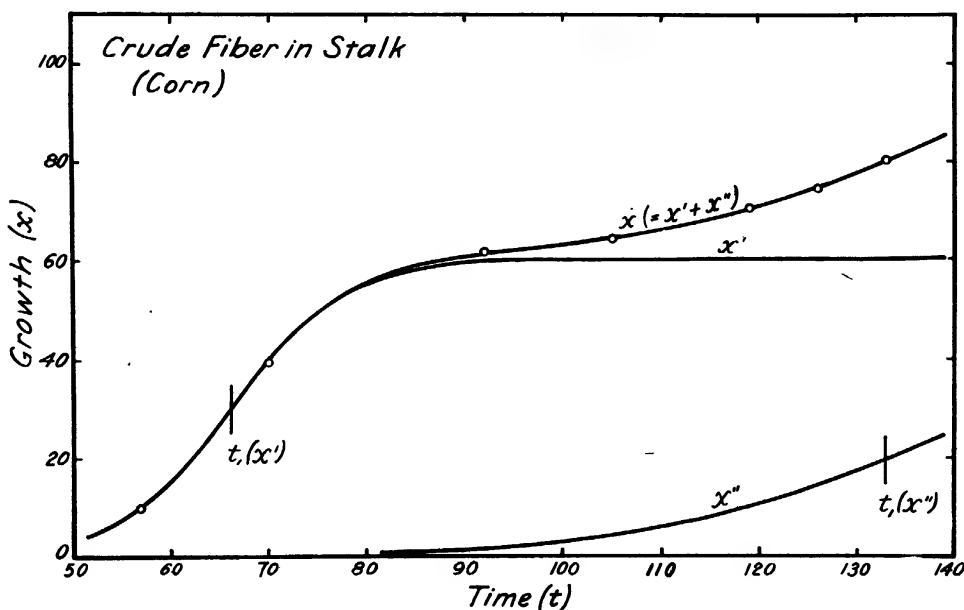


FIG. 5.—Growth of crude fiber in stalk per acre of corn crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 1,710 pounds. Equations to curves:

$$\log \frac{x'}{1030-x'} = 0.075 (t-66.5)$$

$$\log \frac{x''}{680-x''} = 0.033 (t-133)$$

Taken at face value, the data of Figure 5 indicate that the corn is potentially capable of a much greater growth in the reproductive stage than was actually realized, as far as the limitation of the tissues, represented by the present crude fiber data, is concerned. [There is a different relation for the crude-fiber data of the ear (fig. 9).] It is apparent from the data of Figures 4 and 5, that the crude-fiber growth curves of the sunflower and corn are distinctly different. In the first or vegetative cycle the sunflower has a low value of K (0.042) and the corn has a high value (0.075). In the second or reproductive cycle these relations are reversed—that is, the sunflower has a short “grand period” of growth ($K=0.082$) and the corn has a long one ($K=0.033$).

As to the particular location of the growth in crude fiber in the stalk in the second cycle, the data of Figures 4 and 5 do not distinguish. Presumably, the location is in part, at least, in those

organs directly associated with seed development, but included in the stalk data. For the sunflower this would be the head except the seed, and for the corn the husk and shank. Fortunately some data are available in the case of corn showing the proportion of crude fiber in the husk and the remainder of the stalk. Schweitzer¹⁰ gives data which indicate that 19 per cent of the crude fiber of the stalk 138 days after planting was in the husk. Grindley¹¹ found in a crop of corn yielding 100 bushels per acre that 18 per cent of the crude fiber of the stalk was in the husk and silk. According to the equations of Figure 5, at 133 days after planting the second cycle is responsible for 24.8 per cent of the crude fiber of the stalk. Bearing in mind that the shank is not included in the proportions of 19 and 18 per cent, as estimated from Schweitzer's and Grindley's figures, the proportions suggested in Figure 5 from the theoretical treatment of the combined data are consistent with the view that the second growth cycle indicated by the stalk data is quite directly associated with seed development.

GROWTH IN SEED AND EAR

The data just examined indicate that growth in the sunflower and corn crops occurs in two cycles, that the first cycle is associated with the vegetative functions and the second with the reproductive functions. In agreement with this, the development of seed and ear seems to constitute a well defined growth cycle, which, however, in the case of certain constituents is not completed at the death of the stalk. The writers' data on the sunflower seed show that all of the constituents except nitrogen-free extract continued to increase in substantial conformity with the autocatalytic formula. This is in contrast with the behavior of the same constituents of the stalk. In the stalk there is first an accumulation and then a loss in the case of all constituents except crude fiber and aluminum. This loss may be regarded as due to a process of senescence, the reverse of growth.

Properly, one might regard the development of the seed as cycle one, and accordingly A of the seed growth equations is to be divided by the number of seeds per acre to convert the equation to an individual basis. Another growth cycle then ensues at that later time when the seed is provided with the conditions for germination and growth. This cycle also conforms to the autocatalytic formula with reference to the essentially structural materials. With reference to other materials, however, the processes of senescence predominate over those of growth after a time, so that the equation used is not alone adequate to describe the entire changes.

Robertson¹² has analyzed data of Monnier on the growth of the oats plant and has reached the conclusion that the mineral constituents did not follow the autocatalytic law of growth. Considering the sunflower crop as a whole, the writers' data would require the same conclusion with reference not only to the mineral constituents but also to all the other constituents, except crude fiber, crude fat, and aluminum. This condition is due, however, to the senile losses in

¹⁰ SCHWEITZER, P. STUDY OF THE LIFE HISTORY OF CORN AT ITS DIFFERENT PERIODS OF GROWTH. Mo. Agr. Exp. Sta. Bul. 9, 78 p. 1889.

¹¹ Unpublished data, Ill. Agr. Exp. Sta.

¹² ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

the stalk. Growth of ash in the seed conforms very well to the formula, and this is true also for the several elements of the ash.

Some interesting comparisons may be made between the growth curves of the various constituents of the seed. Phosphorus and protein (nitrogen $\times 6.25$) have the same value of K , and protein is directly proportional to the phosphorus of 7 days preceding. The curves for sulphur and fat also have some similarity, in that t_1 is 108 for each, a considerably delayed value as compared with other constituents; and also, for each it is necessary to assume that the growth process was still in active progress at the last observation. The curves for all the data have been worked out to a first approximation,¹³ but there is no need to repeat all of them here. The data for dry matter and crude fiber in seed of sunflower and ear of corn are given, however, for the purpose of certain further comparisons of the two crops on the basis of the growth constants.

DRY MATTER IN SEED AND EAR

The percentage water content of plant tissues varies widely with maturity of the plant. Thus at the time of the first observation on the sunflower seed the water content was 86 per cent and at the last

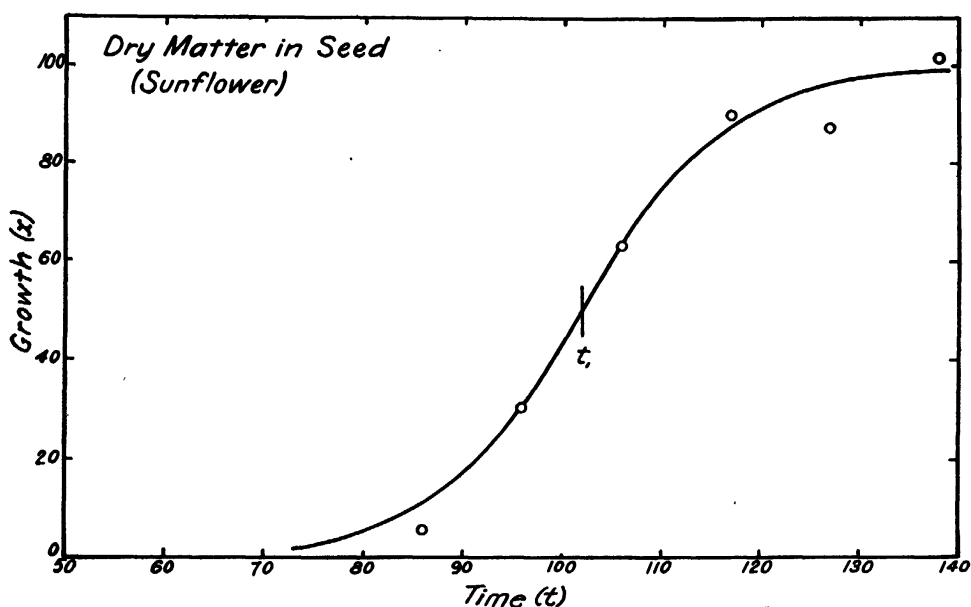


FIG. 6.—Growth of dry matter in seed of sunflower per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 1,450 pounds. Equation to curve:

$$\log \frac{x}{1450-x} = 0.058 (t-102)$$

observation 15 per cent. There are obvious reasons for dealing with the dry matter as a measure of growth. The data for the seed of sunflower and ear of corn are presented in Figures 6 and 7, respectively. The course of growth, as measured by dry matter, conforms satisfactorily to the theoretical considerations. Considering the equations, we find the sunflower with a large value of K (0.058)

¹³ GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407-455, illus. 1925

and the corn with a small value of K (0.038), this agreeing in a general way with the hint offered by the second growth cycle of the stalk as the data were treated in Figures 4 and 5. Another difference in the curves is notable: In treating the growth in dry matter of the ear by the method used it is necessary to assume that growth had not reached its natural limit at the last observation. Such an assumption is not necessary for the sunflower data. This again is consistent with the treatment of the corresponding data of Figures 4 and 5.

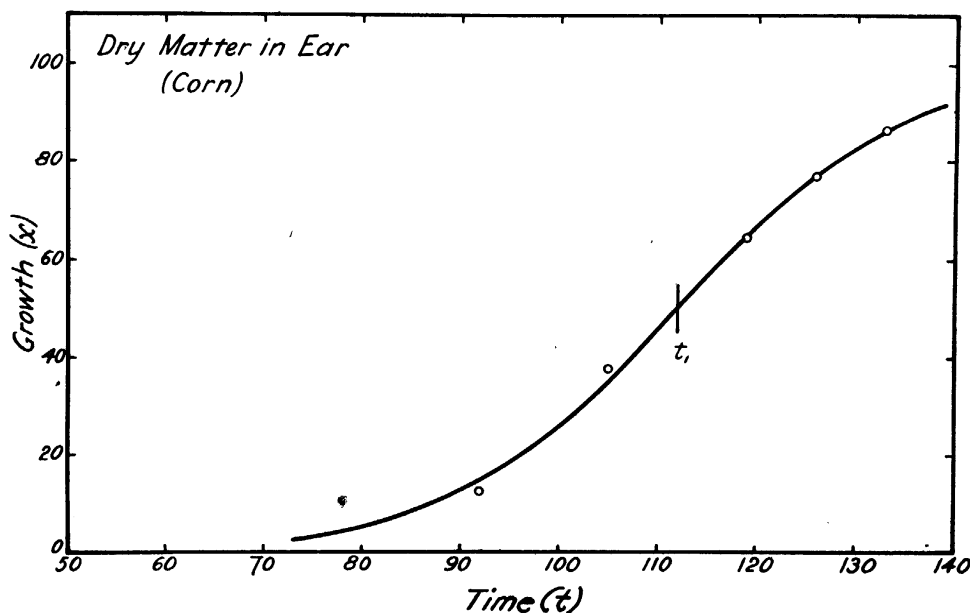


FIG. 7.—Growth of dry matter in ear of corn per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 6,000 pounds. Equation to curve:

$$\log \frac{x}{6000-x} = 0.038 (t-112)$$

CRUDE FIBER IN SEED AND EAR

The course of development of crude fiber in the seed of sunflower (fig. 8) is practically identical with that of dry matter. This is partly a consequence of the fact that crude fiber constitutes a high and fairly constant proportion of the dry matter of the seed throughout the growth cycle. The per cent of crude fiber based on dry matter lies within the range 31 to 39.

The course of crude-fiber development in the ear of corn (fig. 9), however, presents a contrast to that of dry matter. The crude fiber of the ear is largely in the cob (about 85 per cent at maturity). Figure 9 may be presumed, therefore, to represent approximately the growth of the cob. On the basis of interpretation by the equation, the crude-fiber growth cycle is completed, whereas it is not completed in case of dry matter. The apparent completion of the growth cycle of the cob contrasts also with the incomplete second cycle of Figure 5, taken to represent growth of husk and shank. If one were to speculate he might infer that the inherent growth limitations of the cob are a limiting factor in the maximum growth

of the ear. It would seem that any study of the growth of corn from the present standpoint should take account of the growth of the cob as a unit by itself.

GROWTH-CAPACITY CONSTANTS OF SUNFLOWER AND CORN

As a final comparison between the sunflower and corn equations, we may consider the growth-capacity constant A/K . As previously discussed, this constant is theoretically specifically related to the inherent or genetic characteristics of the organism. Since the formula seems to apply equally well to each of several constituents of the crop,

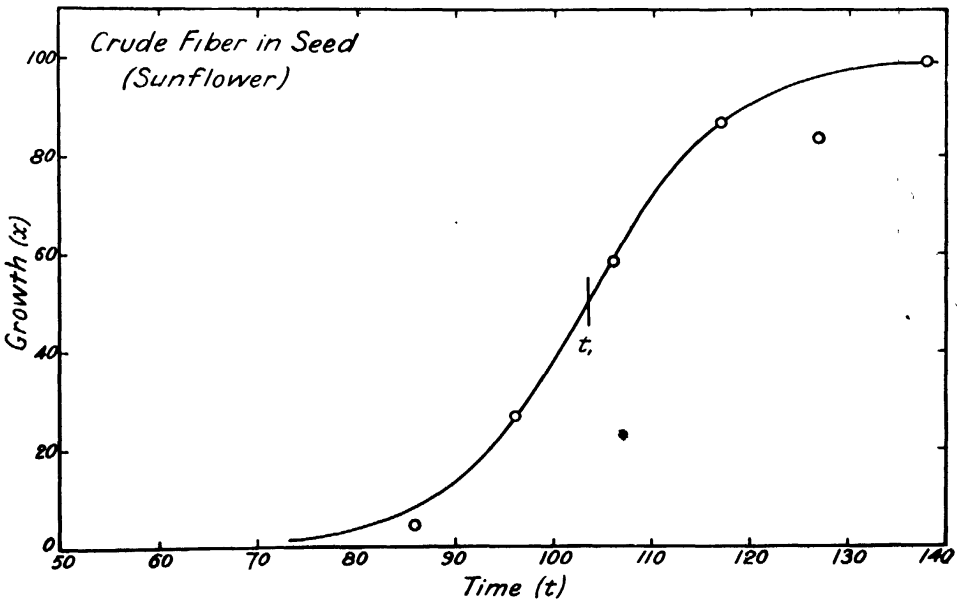


FIG. 8.—Growth of crude fiber in seed of sunflower per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 580 pounds. Equation to curve:

$$\log \frac{x}{580-x} = 0.060 (t-103.5)$$

it may be presumed that the A/K constant is of equal significance for the several constituents. Table I gives the values of the growth capacity constants.

TABLE I.—Comparison of the growth capacity constants of certain constituents of the sunflower and corn crops

Constituent	$A/K \times 10^{-2}$	
	Sunflower	Corn
Crude fiber in stalk *	440	165
Crude fiber in seed or ear	97	74
Dry matter in seed or ear	250	1, 579

* Weighted average of first and second cycles

From this table it appears that the sunflower is preeminently a crude-fiber or roughage crop, while corn is preeminently a grain crop. Comparing crude fiber in the stalk, the sunflower has greater

growth capacity, the constant being 2.7 times that of corn. Comparing dry matter in seed and ear, corn has much the greater capacity, the constant being 6.3 times that of the sunflower. Or we may compare the ratio of dry matter in seed or ear to crude fiber in stalk and find that the ratio for corn is 17 times the ratio for the sunflower. As a cultivated crop, corn is evidently much better adapted to the production of maximum yields of valuable food or feed stuffs than is the sunflower. Breeding and selection may serve to improve the sunflower, but with its apparent inherent handicap of high vegetative and low reproductive development, it has little prospect of ever being a competitor of corn under conditions which permit the growing of corn.

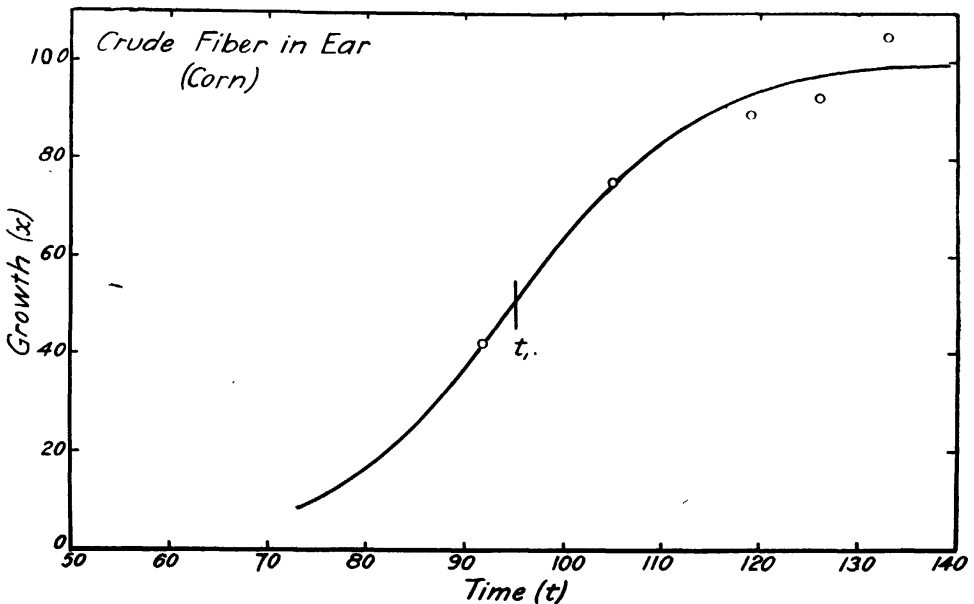


FIG. 9.—Growth of crude fiber in ear of corn per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 350 pounds. Equation to curve:

$$\log \frac{x}{350-x} = 0.047 (t-95)$$

SUMMARY

Data on the growth of the sunflower ¹⁴ and of corn from Bulletin 175, Indiana Agricultural Experiment Station are analyzed (following Robertson ¹⁵) by application of the formula $\log \frac{x}{A-x} = K (t-t_1)$, in which x represents growth accomplished at any time, t . The constants A , K , and A/K of this equation are regarded as of definite significance in connection with crop studies. The constants serve to give numerical expression to the course of growth changes in the crop and should be of value in supplementing the usual data of final crop yield. As between the sunflower and corn crop data studied, pronounced difference is shown, particularly with reference to the vegetative and reproductive growth cycles.

¹⁴ GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407-455, illus. 1925.

¹⁵ JONES, W. J., and HUSTON, H. A. COMPOSITION OF MAIZE AT VARIOUS STAGES OF ITS GROWTH. Ind. Agr. Exp. Sta. Bul. 175: 599-630, illus. 1914.

ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

NET-ENERGY VALUES OF ALFALFA HAY AND ALFALFA MEAL¹

By E. B. FORBES, *Director*, J. AUGUST FRIES, *Assistant Director*, and W. W. BRAMAN, *Associate, Institute of Animal Nutrition, of Pennsylvania State College*

INTRODUCTION

The investigation reported here was undertaken to determine the effect of fine grinding of alfalfa hay, as in the preparation of commercial alfalfa meal, on its net-energy value. Some of the results, computed by an older method, were incorporated in an earlier publication,² but the detailed account of the experiment is first presented in detail here.

The subject of the experiment was a purebred Shorthorn steer 20 months old and having an initial weight of 345 kilograms.

The general plan of the experiment was to feed to this steer, alternately, alfalfa hay and alfalfa meal during six periods (a pair of rations on each of three planes of intake).

The method of experimentation was the same as in the previous net-energy determinations at this institute by the use of the respiration calorimeter.³

PERIODS AND RATIONS

Each feeding period extended over 21 days, of which the first 11 were preliminary and the last 10 the period of the digestion and energy metabolism experiment. On the eighteenth and nineteenth days, 48-hour determinations of the respiration products and the heat production were made by means of the respiration calorimeter. The dates of the several periods, the rations fed, and the average live weight of the animal in each period are shown in Table I.

TABLE I.—Periods, rations, and average live weights

Period	Preliminary period, 1911-12 ^a	Digestion period, 1912	Alfalfa hay	Alfalfa meal	Live weights ^b
			<i>Kg.</i>	<i>Kg.</i>	<i>Kg.</i>
I.....	Dec. 24-Jan. 3.....	Jan. 4-13.....	7.5	-----	348.6
II.....	Jan. 14-24.....	Jan. 25-Feb. 3.....	-----	7.5	348.7
III.....	Feb. 4-14.....	Feb. 15-24.....	6.0	-----	353.7
IV.....	Feb. 25-Mar. 6.....	Mar. 7-16.....	-----	6.0	349.4
V.....	Mar. 17-27.....	Mar. 28-Apr. 6.....	3.5	-----	337.1
VI.....	Apr. 7-17.....	Apr. 18-27.....	-----	3.5	329.4

^a All dates are inclusive.

^b Average of last 6 weights taken at 8 a. m.

¹ Received for publication Apr. 10, 1925; issued January, 1926. This investigation was conducted with the financial cooperation of the Bureau of Animal Industry of the U. S. Department of Agriculture. It was planned, in detail, by H. P. Armsby, and executed by J. A. Fries, W. W. Braman, D. C. Cochran, K. K. Jones, F. W. Christensen, J. W. Parke, and F. C. Dosé.

² ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. Jour. Agr. Research 3: 435-491, illus. 1915.

³ ARMSBY, H. P., and FRIES, J. A. Op. cit.
—, and FRIES, J. A. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 128, 245 p., illus. 1911.

PREPARATION OF THE ALFALFA HAY AND ALFALFA MEAL

The alfalfa hay used was grown in Idaho. From a chosen lot, one half was ground to a fine meal, in the presence of H. P. Armsby, on November 20, 1911, at a commercial mill in Kansas City, Mo. The fine dust which separated from the hay during the grinding was carefully collected and returned to the ground hay. Then both the ground and the unground hay were shipped to the Institute of Animal Nutrition at State College, Pa. The hay was of a bright-green color and excellent quality.

In order to reduce the unground hay to a condition making it convenient to sample for chemical analysis and to handle in the metabolism experiment, it was cut by running through a silage cutter (without the blower). During this process much of the leaf broke up into a fine condition. Of this cut hay, 60 per cent would pass through a $\frac{3}{16}$ -inch-mesh sieve, and about 35 per cent through a $\frac{1}{8}$ -inch-mesh sieve. Comparatively long pieces, however, passed through these sieves lengthwise.

On account of the light, dusty character of the finely ground hay it was necessary to feed it mixed with water, which was added in an amount about equal to the weight of the hay; and in order to keep conditions as nearly as possible the same in all periods, the coarsely cut hay was similarly moistened.

COMPOSITION OF THE FEEDING STUFFS

The composition and energy values of dry matter of the feeds are given in Table II. True protein, as there reported, was determined by the Stutzer method.

TABLE II.—*Dry matter of alfalfa hay and alfalfa meal, and composition of the dry matter*

	Hay				Meal			
	Period I	Period III	Period V	Average	Period II	Period IV	Period VI	Average
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Dry matter.....	88.52	88.67	87.21	88.13	88.98	90.13	90.14	89.75
Ash.....	9.14	8.93	9.12	9.06	9.54	9.09	9.09	9.24
True protein.....	12.70	12.29	12.13	12.37	12.06	11.60	11.60	11.75
Nonprotein.....	2.63	3.05	2.91	2.86	2.82	2.89	2.89	2.87
Crude fiber.....	29.44	30.06	30.82	30.11	30.49	31.44	31.44	31.12
Nitrogen-free extract.....	44.10	43.70	43.11	43.64	43.37	43.07	43.07	43.17
Ether extract.....	1.99	1.97	1.91	1.96	1.72	1.91	1.91	1.85
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Total nitrogen.....	2.59	2.61	2.56	2.59	2.53	2.47	2.47	2.49
True protein nitrogen.....	2.03	1.97	1.94	1.98	1.93	1.86	1.86	1.88
Carbon.....	44.89	44.78	45.27	44.98	44.92	45.17	45.17	45.09
Energy, calories • per kg.....	4,353.80	4,338.30	4,411.80	4,368.00	4,363.70	4,378.70	4,378.70	4,373.70

• Throughout this article the word "calorie" signifies the large, or kilogram, calorie, unless the contrary is specifically stated.

DIGESTIBILITY

The digestibility of the ration was determined in the usual manner. Table III sets forth the digestion coefficients of the hay and of the meal.

TABLE III.—*Digestion coefficients of alfalfa hay and alfalfa meal*

Feed and period	Dry matter	Organic matter	True protein	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Carbon	Energy
Alfalfa hay, dry matter:									
Period I, 6,638.3 grams...	59.9	62.2	71.4	76.6	40.6	74.0	14.4	58.7	58.8
Period III, 5,320.2 grams...	60.4	61.7	67.7	74.1	45.0	70.7	19.4	59.0	58.6
Period V, 3,052.4 grams...	63.7	64.4	71.0	74.4	47.3	73.6	35.2	63.0	62.3
Average.....	61.3	62.8	70.0	75.0	44.3	72.8	23.0	60.2	59.9
Alfalfa meal, dry matter:									
Period II, 6,670.7 grams...	58.2	59.4	66.1	72.7	38.0	72.2	2.4	57.5	56.8
Period IV, 5,407.8 grams...	57.8	59.5	66.3	72.5	37.2	72.5	31.1	56.5	56.6
Period VI, 3,154.9 grams...	61.3	61.7	69.3	75.8	43.1	71.9	35.1	60.5	60.5
Average.....	59.1	60.2	67.2	73.7	39.4	72.2	22.9	58.2	58.0

In all periods the hay was appreciably more completely digested than was the meal. This difference prevailed consistently, without exception, in regard to dry matter, organic matter, true protein, crude protein, and crude fiber. There were exceptions to this order, in some cases, with reference to nitrogen-free extract and ether extract.

Naturally these differences in the digestibility of alfalfa hay and alfalfa meal depended on the meal being used as the sole roughage.

While the writers do not have positive evidence to explain this difference, it seems proper to suggest a probable explanation. In view of their understanding of the conditions which determine the course of the food in the alimentary tract of ruminants, especially the influence of fineness of grinding on the passage of the food when swallowed, into the first, second, and third stomachs, and its tendency therefore, to suppress rumination, it seems likely to the writers that the lower digestibility of the meal was due to its having been swallowed, in part at least, past the paunch, and, consequently, to having escaped to this extent the usual prolonged soaking and fermentation in that organ, and the subsequent regurgitation and remastication, while normal conditions prevailed in the case of the coarsely cut hay.

The higher apparent digestibility of the hay and the meal in Periods V and VI than in Periods I to IV is not unusual in periods of very low feed intake, this being due in part to the slower passage of the feed through the alimentary tract and increased fermentation per unit of weight of feed. In these periods of low feed intake there was also evidence of slight abnormality in the condition of the subject, perhaps as a result of slow movement of alimentary residues, the indications being irregular drinking, abundant sediment in the urine, mucus in the feces, and foul odor of urine and feces. The daily fecal elimination, however, was not unduly variable, not sufficiently so to warrant a conclusion that fecal elimination was so delayed as seriously to compromise the accuracy of the digestion coefficients.

THE BALANCE OF MATTER

From the data for balance of matter, in Table IV, it will be noted that with decreased feed intake there was marked decrease in methane elimination, but when we relate the amount of the methane to the amount of the feed we see that with decrease in feed intake there was a slight increase in the methane production per kilogram of dry matter of feed. Thus in Periods I, III, and V, with feed intake of 6,638, 5,320, and 3,052 grams, respectively, the methane production per kilogram of dry matter of feed was 18.769, 19.453, and 21.871 grams, respectively.

TABLE IV.—Balance of matter and energy per day and head

	Period I					Period III				
	Dry matter	Water	Nitrogen	Carbon	Energy	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Alfalfa hay.....	6,638.3	6,332.0	172.0	2,980.2	28,921.1	5,320.2	4,675.0	139.1	2,382.4	23,178.5
Water.....		13,295.0					16,100.0			
Total.....	6,638.3	19,627.0	172.0	2,980.2	28,921.1	5,320.2	20,775.0	139.1	2,382.4	23,178.5
Outgo, Group I:										
Feces.....	2,663.2	9,710.1	40.2	1,230.9	11,924.3	2,104.8	9,493.0	36.1	977.5	9,601.8
Urine.....	723.0	8,316.3	115.2	166.8	1,819.5	561.6	5,366.2	93.7	137.5	1,420.1
Methane.....	137.95			103.2	1,840.8	112.9			84.5	1,506.5
Total.....					15,584.6					12,528.4
Metabolizable.....					13,336.5					10,650.1
Outgo, Group II:										
Hair and brushings.....	12.7	1.1	1.0	5.5	60.7	12.7	1.1		5.5	60.7
Carbon dioxide.....	4,719.0			1,286.9		4,054.0				
Water vapor.....		4,587.5					3,740.2			
Body balances:										
Water.....		-2,988.0					+2,174.5			
Protein.....	+93.0		+15.5	+48.9	+414.6	+49.8		+8.3	+26.2	+221.1
Fat.....	+180.5			+138.0	+1,714.3	+59.7			+45.7	+567.2
Computed heat.....					11,146.9					9,801.1
Observed heat.....					11,088.5					9,723.3
	Period V					Period II				
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Income:										
Alfalfa hay.....	3,052.4	4,458.0	78.1	1,381.8	13,298.4	6,670.7	7,846.0	168.7	2,996.7	29,175.6
Water.....		6,975.0					16,240.0			
Total.....	3,052.4	11,433.0	78.1	1,381.8	13,298.4	6,670.7	24,086.0	168.7	2,996.7	29,175.6
Outgo, Group I:										
Feces.....	1,108.5	4,938.0	20.0	511.3	5,010.4	2,786.9	11,798.0	46.0	1,273.0	12,600.0
Urine.....	362.1	4,117.6	65.3	98.1	865.2	677.3	7,357.5	107.1	161.5	1,666.6
Methane.....	68.4			51.2	912.8	125.2			93.7	1,670.7
Total.....					6,788.4					15,937.3
Metabolizable.....					6,510.0					13,238.3
Outgo, Group III:										
Hair and brushings.....	12.7	1.1	1.0	5.5	60.7	12.7	1.1	1.0	5.5	60.7
Carbon dioxide.....	2,850.8			777.4		4,593.3			1,252.6	
Water vapor.....		2,509.1					4,547.1			
Body balances:										
Water.....		-132.8					+382.3			
Protein.....	-49.2		-8.2	-25.8	-219.3	+87.6		+14.6	+46.0	+390.5
Fat.....	-46.9			-35.9	-445.6	+215.0			+164.4	+2,042.1
Computed heat.....					7,114.2					10,745.0
Observed heat.....					7,303.7					10,886.0

* Corrected to nitrogen equilibrium.

† Alfalfa meal.

TABLE IV.—Balance of matter and energy per day and head—Continued

	Period IV					Period VI				
	Dry matter	Water	Nitro- gen	Carbon	Energy	Dry matter	Water	Nitro- gen	Carbon	Energy
Income:	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
Alfalfa meal.....	5,407.8	4,607.0	133.6	2,442.8	23,652.1	3,154.9	4,346.0	78.0	1,425.1	13,798.6
Water.....	-----	12,000.0	-----	-----	-----	-----	4,250.0	-----	-----	-----
Total.....	5,407.8	16,607.0	133.6	2,442.8	23,652.1	3,154.9	8,596.0	78.0	1,425.1	13,798.6
Outgo, Group I:										
Feces.....	2,280.5	9,168.0	36.8	1,062.8	10,255.5	1,221.3	4,583.0	18.9	563.3	5,444.8
Urine.....	524.5	5,804.4	90.7	131.4	1,331.6	288.1	3,752.3	58.3	102.6	846.7
Methane.....	105.2	-----	-----	78.7	1,403.8	69.0	-----	-----	51.6	920.1
Total.....	-----	-----	-----	-----	12,990.9	-----	-----	-----	-----	7,211.6
Metabolizable.....	-----	-----	-----	-----	10,661.2	-----	-----	-----	-----	6,587.0
Outgo, Group II:										
Hair and brush- ings.....	12.7	1.1	1.0	5.5	60.7	12.7	1.1	1.0	5.5	60.7
Carbon dioxide ..	4,004.1	-----	-----	1,092.0	-----	2,694.7	-----	-----	734.9	-----
Water vapor.....	-----	3,791.5	-----	-----	-----	-----	2,391.9	-----	-----	-----
Body balances:										
Water.....	-----	-2,158.0	-----	-----	-----	-----	-2,132.3	-----	-----	-----
Protein.....	+30.6	-----	+5.1	+16.1	+136.4	-1.2	-----	0.2	0.6	-5.3
Fat.....	+73.7	-----	-----	+56.3	+699.7	-42.1	-----	-----	32.2	-399.5
Computed heat.....	-----	-----	-----	-----	9,764.4	-----	-----	-----	-----	6,931.1
Observed heat.....	-----	-----	-----	-----	9,697.6	-----	-----	-----	-----	6,795.0

• Corrected to nitrogen equipment.

The balances of protein and fat reflect the differing intake of feed in a generally consistent manner, but the losses of protein in Periods V and VI are not as nearly alike as might have been anticipated.

The data representing the balance of energy in the several periods are presented, for reference, in Table IV, but the significance of the figures, in comparison, is more readily apparant in another arrangement, and will be discussed in connection with Table VII.

HEAT PRODUCTION

The observed heat production (Table V) is the measured heat emission corrected for gain or loss of actual heat in body gain or loss and for loss of heat in excreta.

TABLE V.—Observed and computed average daily heat production

	Observed heat	Computed heat	Apparent error	Computed, as per cent of observed
	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	
Period I.....	11,088.5	11,146.9	+58.4	100.5
Period III.....	9,723.3	9,801.1	+77.8	100.8
Period V.....	7,303.7	7,114.2	-189.5	97.4
Period II.....	10,886.0	10,745.0	-141.0	98.7
Period IV.....	9,697.6	9,764.4	+66.8	100.7
Period VI.....	6,795.0	6,931.1	+136.1	102.0

The accuracy of this observed heat production may be confirmed by indirect computation—subtracting from the gross energy of the feed the determined energy of the excreta (urine, feces, and combustible gases), and the energy of the body gains, as computed from the balances of carbon and nitrogen. Such a comparison of

the observed and the computed heat production is made in Table V and shows the measurement of the heat production to have been satisfactory.

CORRECTION OF OBSERVED HEAT PRODUCTION

Preparatory to use in the computation of the net energy of a feed it is necessary to apply a correction of the observed heat production to compensate for inequality of time spent by the animal in the standing and the lying positions, a standard day of 12 hours standing and 12 hours lying having been arbitrarily adopted as a feature of the experimental routine.

This correction was made in accord with the recently published method of Fries and Kriss,⁴ the details of which will not be repeated here. The method of this correction and the corrected data are as indicated in Table VI.

TABLE VI.—Observed heat production corrected to 12 hours standing and 12 hours lying

Period	Observed daily heat production	Hours standing	Difference from 12 hours	Live weight	Hourly correction for position	Correction to standard day	Corrected heat production
	<i>Calories</i>			<i>Kilograms</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>
I.....	11,088.5	10.13	1.87	348.6	24.1	45.1	11,133.6
III.....	9,723.3	7.05	4.95	353.7	24.1	119.3	9,842.6
V.....	7,303.7	8.26	3.74	337.1	24.1	90.1	7,393.8
II.....	10,886.0	9.50	2.50	348.7	24.1	60.3	10,946.3
IV.....	9,697.6	9.14	2.86	349.4	24.1	68.9	9,766.5
VI.....	6,795.0	8.98	3.02	329.4	24.1	72.8	6,867.8

METABOLIZABLE ENERGY, HEAT PRODUCTION, AND ENERGY GAIN

In Table VII it is seen that the metabolizable energy varies, in general consistently with the total dry matter, but that as related to the kilograms of dry matter the metabolizable energy of the hay is slightly higher than that of the meal, in harmony with the previously noted difference in digestibility; and that the metabolizable energy on the two higher planes of feed intake, with both feeds, is significantly lower than on the lowest plane of intake. On the two higher planes of intake the metabolizable energy per kilogram of dry matter of both hay and meal agrees very well.

TABLE VII.—Dry matter, metabolizable energy, heat production, and energy gain

Feeding stuff and period No.	Dry matter eaten	Metabolizable energy, total	Metabolizable energy per kilogram dry matter	Heat production corrected to standard day	Gain of energy
	<i>Kilograms</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>
Alfalfa hay:					
Period I.....	6.638	13,337	2,009	11,134	+2,203
Period III.....	5.320	10,650	2,002	9,843	+807
Period V.....	3.052	6,510	2,133	7,394	—884
Alfalfa meal:					
Period II.....	6.671	13,238	1,985	10,946	+2,292
Period IV.....	5.408	10,661	1,971	9,767	+894
Period VI.....	3.155	6,587	2,088	6,868	—281

⁴ FRIES, J. A., and KRISS, M. METABOLISM OF CATTLE DURING STANDING AND LYING. Amer. Jour Physiol. 71: 60-83. 1924.

The figures for heat production, however, when considered in relation to the amount of feed, are less satisfactory, that for Period VI being especially low, with the result that the loss of energy in this period was correspondingly low.

THE HEAT INCREMENT

The heat increment (Table VIII) is the increased heat production due to an increase in the amount of feed, and is expressed, in relation to the kilograms of dry matter of the increase in feed, as the heat increment.

TABLE VIII.—Heat increment per kilogram of dry matter

Feeding stuff	Periods compared	Heat incre- ment per kilogram dry matter	Feeding stuff	Periods compared	Heat incre- ment per kilogram dry matter
		Calories			Calories
Alfalfa hay.....	I and III.....	979	Alfalfa meal.....	II and IV.....	934
Do.....	III and V.....	1,080	Do.....	IV and VI.....	1,287
Do.....	I and V.....	1,043	Do.....	II and VI.....	1,160
Average.....		1,034	Average.....		1,127

With both the hay and the meal there are two high and one low heat-increment values, the high values being those affected by heat production in Periods V and VI, the two involving minimum feed intake.

THE MAINTENANCE REQUIREMENT

The maintenance requirement of energy is computed for each period by subtracting from the heat production the product of the heat-increment value multiplied by the kilograms of dry matter of the feed, as indicated in Table IX.

TABLE IX.—Maintenance requirement of net energy

Period No.	Dry matter eaten		Average heat incre- ment per kilogram of dry matter	Total heat increment of ration	Total heat production	Net energy required for main- tenance
	Alfalfa hay	Alfalfa meal				
Treatment A:	Kilograms	Kilograms	Calories	Calories	Calories	Calories
I.....	6.6383		1,034	6,864	11,134	4,270
II.....		6.6707	1,127	7,518	10,946	3,428
III.....	5.3203		1,034	5,501	9,843	4,342
IV.....		5.4078	1,127	6,095	9,767	3,672
V.....	3.0524		1,034	3,156	7,394	4,238
VI.....		3.1549	1,127	3,556	6,868	3,312
Average.....						3,877
Treatment B:						
I.....	6.6383		979	6,499	11,134	4,635
III.....	5.3203		979	5,209	9,843	
II.....		6.6707	934	6,230	10,946	4,716
IV.....		5.4078	934	5,051	9,767	
Average.....						4,676

It will be noted that two treatments of the experimental data are given in this table, treatment A representing the usual procedure, and treatment B the same except for the elimination of the data for Periods V and VI, there being, in the writers' opinion, sufficient warrant for pointing out in this way the influence of the data for Periods V and VI, but not sufficient, at this time, to warrant excluding these data from consideration.

The influence of the high heat production in Periods V and VI is reflected, in treatment A, in (1) high average heat-increment values in all periods, but especially in Periods II, IV, and VI, and (2) abnormally low net energy values for maintenance in all periods, but especially in Periods II, IV, and VI. Obviously these latter values are wrong.

Treatment B, exhibiting the computation of the maintenance requirement with the data for Periods V and VI excluded, is consistent within itself and with what is known of the maintenance requirement of steers.

NET-ENERGY VALUES

The net-energy value of the feeds is computed by adding the body gain of energy to the maintenance requirement, or subtracting the body loss of energy from the same. This gives the total amount of net energy for the feed of each period, and this value divided by the kilograms of dry feed involved gives the net energy per kilogram of dry matter (Table X).

TABLE X.—*Net-energy values of alfalfa hay and alfalfa meal*

Feeding stuff and period No.	Dry matter eaten	Metabolizable energy	Average maintenance requirement of net energy	Body gain of energy	Total net energy of ration	Net energy per kilogram of dry matter	Utilization of metabolizable energy
Treatment A:							
Alfalfa hay—	<i>Kg.</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Per cent</i>
I.....	6. 6383	13, 337	3, 877	+2, 203	6, 080	916	45. 59
III.....	5. 3202	10, 650	3, 877	+807	4, 684	880	43. 98
V.....	3. 0524	6, 510	3, 877	—884	2, 993	981	45. 98
Average.....						926	45. 18
Alfalfa meal—							
II.....	6. 6707	13, 238	3, 877	+2, 292	6, 169	925	46. 60
IV.....	5. 4078	10, 661	3, 877	+894	4, 771	882	44. 75
VI.....	3. 1549	6, 587	3, 877	—281	3, 596	1, 140	54. 59
Average.....						982	48. 65
Treatment B:							
Alfalfa hay—							
I.....	6. 6383	13, 337	4, 676	+2, 203	6, 879	1, 036	51. 58
III.....	5. 3202	10, 650	4, 676	+807	5, 483	1, 031	51. 48
Average.....						1, 034	51. 53
Alfalfa meal—							
II.....	6. 6707	13, 238	4, 676	+2, 292	6, 968	1, 045	52. 64
IV.....	5. 4078	10, 661	4, 676	+894	5, 570	1, 030	52. 25
Average.....						1, 038	52. 45

A figure of similar significance, expressing the percentage of utilization of the metabolizable energy, is obtained by dividing the net energy by the metabolizable energy.

Treatment A exhibits considerable diversity in the net energy values for both feeds, but especially for the alfalfa meal—a diversity which does not permit of a distinction between the values of the two feeds; while treatment B gives very closely concordant values and virtually identical figures for the hay and for the meal.

This indication that there is no difference in the nutritive values of the two feeds is in harmony with the conclusion of Fries and Kriss,⁵ that the heat production due to the increased muscular activity during the intake of feed and water is normally less in amount than that required to bring the temperature of the ingested feed and water up to that of the body.

SUMMARY

The net-energy value of alfalfa hay of excellent quality is essentially the same when coarsely cut, in a silage cutter, as when finely ground into a meal.

Probably due to difference in the course of digestion, especially with reference to rumination, the finely ground hay was 2.2 per cent less digestible, on a dry-matter basis, than was the coarsely cut hay.

⁵ FRIES, J. A., and KRISS, M. Op. cit.

THE VALUE OF LITMUS, BROM-CRESOL PURPLE, AND JANUS-GREEN MILK IN A STUDY OF THE NODULE ORGANISMS OF LEGUMINOSAE¹

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INTRODUCTION

The value of milk in the study of the nodule bacteria of legumes has been shown by Löhnis and Hansen.² They found that in milk, organisms with peritrichous flagella from alfalfa, sweet clover, clover, pea, vetch, lupine, and bean formed a clear serum zone on top 2 to 5 mm. thick, while the milk below remained nearly unchanged; and that organisms of the second group, such as cowpea, peanut, lima bean, and soy bean, which have only one flagellum, formed no serum zone but that later there was a slow digestion of the milk. They also pointed out the value of milk in distinguishing *Bacillus radiobacter* from nodule bacteria of legumes.

The interesting reaction of the nodule bacteria in milk suggested the possibility of improving plain milk by the addition of some indicator, which would show reduction and change in reaction. In an attempt to improve the medium, litmus, brom-cresol purple, and Janus-green milk media were prepared and their value compared with plain milk.

Only fresh skim milk was used as culture medium. This was sterilized in an Arnold steamer at 100° C. for 30 minutes on four successive days. Aqueous solutions of the indicators were sterilized separately and added to the milk. For a comparison of plain and litmus milk four parallel tubes of each were inoculated with the same culture, while with brom-cresol purple and Janus green only two tubes of each culture were used. Pure cultures of nodule bacteria from pea, vetch, clover, garden bean, lima bean, cowpea, soy bean, alfalfa, and sweet clover were used. All cultures were incubated at 28° C.

Of the three indicators, litmus proved by far the most valuable. Brom-cresol purple and Janus green failed to bring out any differences not shown by litmus. Brom-cresol purple showed changes in reaction, while Janus green showed a reduction. As Janus green remains reduced permanently it is of less diagnostic value than litmus. Brom-cresol purple was not reduced by any of the nodule organisms. This indicator retarded the growth of the organisms, in some cases completely. High concentrations of litmus in milk had no noticeable inhibitive effect on the growth of these organisms, and they showed changes in reaction equally as well as brom-cresol purple, and in indicating reduction they were as satisfactory as Janus green:

After one to three weeks most of the cultures showed an alkaline reaction in brom-cresol-purple milk. However, after five weeks some

¹ Received for publication May 6, 1925; issued January, 1926. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

² LÖHNIS, F., and HANSEN, R. NODULE BACTERIA OF LEGUMINOUS PLANTS. Jour. Agr. Research 20: 543-556, illus. 1921.

of the alfalfa and sweet clover strains produced an acid reaction. Some of the cultures showed no change during eight weeks. Perhaps this was due to a failure of the organisms to grow. These data are shown in Table I.

TABLE I.—*Characteristics of nodule bacteria in milk, with various indicators, after five weeks at 28° C.*

Strain and No.	Plain milk	Litmus milk		Brom-cresol purple milk reaction
		Reaction	Reduction	
Sweet clover, 110, 111, 112.....	Serum zone.....	Acid.....	At bottom.....	Acid.
Alfalfa, 106, 107, 108.....	do.....	do.....	do.....	Do.
Alfalfa, 100, 103, 104.....	do.....	do.....	do.....	No change.
Alfalfa, 101, 102, 105.....	do.....	do.....	do.....	Slightly alkaline.
Clover, 121, 122.....	do.....	do.....	Throughout.....	Very alkaline.
Clover, 123, 124, 125.....	do.....	Alkaline.....	None.....	Do.
Garden pea, 130.....	do.....	Very alkaline.....	Slight.....	Do.
Garden pea, 132.....	do.....	do.....	None.....	Slightly alkaline.
Garden pea, 131.....	do.....	do.....	Throughout.....	Very alkaline.
Vetch, 134.....	do.....	Very alkaline.....	None.....	Alkaline.
Garden bean, 140.....	do.....	do.....	do.....	No change.
Cowpea, 143.....	None.....	do.....	do.....	Alkaline.
Lima bean, 142.....	do.....	do.....	do.....	No change.
Soy bean, 161, 164, 166.....	do.....	do.....	do.....	No change.
Soy bean, 160, 162, 163, 165.....	do.....	do.....	do.....	Very alkaline.

Most of the organisms reduced Janus green to a permanent pink color in from one to three weeks, as shown in Table II. Some cultures reduced this indicator only at the bottom, while others reduced it throughout. Soy-bean, cowpea, and lima-bean nodule organisms were least active in reducing the indicator. Three strains of soy-bean nodule organisms produced no change within 39 days. Very little, if any, change in reaction was ever noted in the Janus-green culture.

TABLE II.—*Effect of the growth of the nodule bacteria on Janus-green milk (duplicate tubes)*

Strain and No.	Changes produced after—	
	7 days	39 days
Sweet clover, 110, 111, 112.....	Pink at bottom.....	Pink.
Alfalfa, 100.....	Pink.....	Do.
Alfalfa, 106, 107, 108.....	Pink at bottom.....	Do.
Alfalfa, 103.....	do.....	Pink at bottom.
Alfalfa, 101, 102, 104, 105.....	No change.....	Do.
Clover, 121.....	Light pink.....	Pink.
Clover, 123.....	Whitish green.....	Do.
Clover, 124.....	Pink.....	Do.
Clover, 125.....	Light pink.....	Do.
Garden pea, 131.....	Pink.....	Do.
Garden pea, 132.....	No change.....	Do.
Vetch, 134.....	Pink.....	Do.
Garden bean, 140.....	do.....	Do.
Lima bean, 142.....	No change.....	Pink at bottom.
Soy bean, 161, 164, 166.....	do.....	No change.
Soy bean, 160, 162, 163.....	do.....	Pink at bottom.
Soy bean, 165.....	do.....	Pink.

The results obtained with litmus milk were more specific and were superior to those obtained with plain milk. As will be seen in Table I, litmus milk not only showed all the characteristics brought out by plain milk, but in addition showed changes in reaction and reduction.

According to change in reaction, the cultures fall into two groups: Acid production with strong reduction, and alkaline production with little or no reduction.

The cultures from alfalfa and sweet-clover nodules were the only ones that showed an acid reaction. Within the first few days after inoculation the organisms produced an alkaline reaction in milk, but later they began to reduce the litmus, and still later the milk began to turn pink. The rate of acid production was much faster with freshly isolated strains. The tubes of milk usually remained reduced at the bottom, while the upper layer was pink. Some cultures kept the litmus reduced completely below the serum zone.

The cultures of the second group—which included pea, vetch, clover, bean, lupine, cowpea, lima-bean and soy-bean organisms—produced alkaline reactions (Table I). The milk cultures showed a change in reaction within a few days after inoculation. The most marked changes were produced by soy-bean, lima-bean, cowpea, and pea cultures. Some clover and pea strains reduced the litmus completely after about two weeks. These were cultures which had been cultivated in the laboratory for a number of years. Other clover, pea, and bean strains reduced the litmus slightly, usually at the bottom of the tube. Cultures of cowpea, lima bean, and soy bean did not reduce the litmus. A film growth over the surface was formed by some cultures of clover, pea, and vetch. This film formation was especially noticeable with strains which showed a marked reduction of the litmus.

The characteristic reaction of *Bacillus radiobacter* is shown quite as well in litmus milk as in milk without the indicator.

The effect of the growth of these cultures on the hydrogen-ion concentration of the milk after 47 days was studied. The determinations reported below were made by the electrometric method:

	PH
Uninoculated.....	6.55
Alfalfa, 108.....	5.18
Do. 109.....	5.31
Soy bean, 164.....	7.75
Do. 168.....	8.37
Lupine, 150.....	7.55
Lima bean, 142.....	7.85
Cowpea, 143.....	8.08

The alkaline reaction produced by the growth of soy-bean, cowpea, and lima-bean nodule organisms in milk probably accounts for the liquefying effect on the milk casein noted by Löhnis and Hansen.³ These investigators noted a slow digestion of the casein by these organisms after a long time. Preliminary experiments by the writer indicate that the nodule organisms have very little, if any, effect on the milk casein. However, the alkaline reactions produced by soy-bean, cowpea, and lima-bean nodule organisms, as shown above, are sufficient to cause a slow dissolving of the milk casein, with a resulting decrease in opacity of the milk.

A serum zone was formed by the alfalfa, sweet-clover, clover, pea, vetch, and bean cultures. Clover, pea, and vetch cultures would often produce a serum zone in two or three days, while some strains of alfalfa, sweet-clover and bean organisms would often require

³ Op. cit.

from two to four weeks. The cowpea, lima-bean, and soy-bean cultures did not form a serum zone. The rate of serum-zone formation is influenced greatly by the amount of inoculum.

The behavior of the various cultures of nodule-forming bacteria in litmus milk was of considerable value in separating strains. Differences were noted in rate of serum-zone formation, depth of zone, amount of reduction, and in change of reaction among organisms of a single cross-inoculating group. Probably the ability of these organisms to change the reaction of the medium in which they grow is an adaptation to their natural environment and has some relation to their life functions.

CONCLUSIONS

The litmus milk brings out characteristics of the nodule bacteria not shown by plain milk. On the basis of reaction and reduction, it separates these organisms into two groups. This separation is more marked with freshly isolated cultures than with cultures carried in the laboratory for a long time.



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CEREAL INVESTIGATIONS.

A CHEMICAL STUDY OF THE FLESH OF EMACIATED CATTLE¹

By RALPH HOAGLAND, *Biochemist*, and WILMER C. POWICK, *Associate Biochemist, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

WORK OF OTHER INVESTIGATORS

Emaciation in cattle, in the absence of recognizable disease, may be regarded as due to lack of feed, to malnutrition, or to a combination of these factors. So far as this condition is caused by lack of feed, the work of Trowbridge, Moulton, and Haigh (13)² is of interest. In their experiments seven thrifty, fat, yearling steers, as nearly uniform as it was practicable to obtain them, were used. One was slaughtered as a check at the beginning of the test. The others were fed a standard ration throughout the experiment in such quantities that two of them remained at approximately constant weight, two gained, and the other two lost approximately one-half pound each a day. The "maintenance" animals were slaughtered at the end of 6 and 12 months, respectively, the "submaintenance" animals at the end of 6 and 11 months, respectively; one of the "supermaintenance" steers at the end of 12 months, and the other discarded after 16 months. Of the steers receiving the submaintenance ration, the one slaughtered at the end of 6 months was in very poor condition and the other, slaughtered at the end of 11 months, was so extremely emaciated that it could scarcely have survived another month. In Table I are shown the apparent percentage changes in weight of the various parts and organs of the experimental animals, as calculated from the days found in the report.

It seems desirable to call particular attention to the data from steers Nos. 591 and 592, which were fed the submaintenance rations. The most noticeable thing is the relatively large decrease in the weights of the liver, pancreas, spleen, and thymus of each steer. The carcass weight of each steer also decreased very considerably, particularly that of steer No. 592, which was fed 11 months. The blood, kidneys, and intestines of these steers also decreased materially in weight, but the heart lost only slightly. On the other hand, the lungs of the submaintenance steers gained slightly in weight, and the brain and spinal cord made rather large gains.

The effect of the submaintenance ration on the deposits of fat in the carcasses of the two steers was pronounced. Steer No. 591 lost more than two-thirds and steer No. 592 more than 95 per cent of its adipose tissue during the feeding periods of 6 and 11 months, respectively. The fat content of the skeleton of steer No. 591 increased materially during the feeding period, but steer No. 592 lost

¹ Received for publication Apr. 15, 1925; issued January, 1926.

² Reference is made by number (italic) to "Literature cited," p. 1013.

more than 75 per cent of the fat present at the beginning of the test. The marrow of the hollow bones of steer No. 592, which was fed the submaintenance ration for 11 months, contained practically no fat.

TABLE I.—*Effect of fattening, maintenance, and submaintenance rations on development of steers* ^a

Part of organ	Maintenance steers		Submaintenance steers		Fat steer
	No. 597 (6 months)	No. 595 (12 months)	No. 591 (6 months)	No. 592 (11 months)	No. 593 (6 months)
	<i>Per cent change</i>	<i>Per cent change</i>	<i>Per cent change</i>	<i>Per cent change</i>	<i>Per cent change</i>
Empty weight.....	+0. 19	—1. 60	—18. 67	—30. 67	+11. 40
Carcass.....	—1. 45	—1. 83	—21. 83	—36. 77	+13. 39
Blood.....	—4. 90	+7. 72	—17. 47	—16. 71	—2. 80
Heart (muscle).....	—11. 99	+14. 64	—6. 56	—5. 59	+2. 59
Brain.....	—3. 99	+17. 08	+19. 83	+16. 34	+1. 88
Spinal cord.....	+24. 14	+33. 68	+36. 31	+39. 42	+18. 27
Liver.....	—40. 78	—29. 29	—57. 90	—52. 75	—24. 42
Spleen.....	—17. 62	+19. 09	—27. 79	—41. 86	+5. 75
Kidneys.....	—17. 91	+1. 64	—23. 17	—6. 32	—4. 47
Pancreas.....	—22. 82	+41. 03	—43. 80	—44. 48	—4. 13
Thymus.....	+12. 25	—21. 21	—40. 69	—83. 58	+35. 04
Stomachs.....	+15. 78	+33. 11	+5. 52	—2. 37	+45. 49
Intestines.....	—14. 37	+2. 93	—21. 20	—7. 11	+7. 20
Lungs and trachea.....	—6. 67	+1. 70	+9. 05	+8. 68	+2. 36

^a This table has been prepared by recalculating the data presented in Table 21 of the report by Trowbridge, Moulton, and Haigh (13, p. 64–66) so as to show the percentage change in weight of the different parts and organs of the animals during the feeding periods. The control animal slaughtered at the beginning of the test was used as the standard of reference.

The data in Table I show very clearly the pronounced effects of undernutrition on the development of the different parts and organs of the steer. In view of the marked losses suffered by most of the organs and by the carcass as a whole, it is noteworthy that some organs maintained their weight or even increased it.

In another publication Moulton (11) has reported the results of a chemical study of the flesh, blood, and liver of the same lot of steers, and in summarizing his results states, in part:

Inanition or partial starvation does not cause a watery muscular tissue. Fat is almost entirely resorbed, while glycogen apparently is not * * *. The phosphorus content of the flesh is reduced 10 to 15 per cent.

The liver has a somewhat higher water content in the starved animal, accompanied by a high nitrogen content. * * * The glycogen content is not depleted.

The blood has more water and less nitrogen in the fasted steer.

The muscle fibers have become very much smaller, but still are functioning muscle fibers.

Feder (2) determined the composition of the flesh of four emaciated cattle, but since two of the animals were affected with recognized diseases the analyses of these carcasses will not be discussed. The composition of the flesh of the other two emaciated cattle is shown in Table II.

TABLE II.—*Composition of flesh of emaciated cattle*
[Results are expressed in terms of percentages of fresh material] ^a

Description of sample	Moisture	Fat	Ash	Nonfatty organic matter	Ratio of nonfatty organic matter to moisture
1. Muscle from shoulder of 7-year-old cow which had become exhausted during transit on railway. Badly emaciated. No pathological changes in organs.....	<i>Per cent</i> 78. 95	<i>Per cent</i> 1. 33	<i>Per cent</i> 1. 14	<i>Per cent</i> 18. 58	1:4. 25
2. Composite sample of very watery flesh from an animal so badly emaciated that practically all fat had been used up. No pathological changes in organs. Meat sold on "Freibank".....	80. 78	0. 35	0. 98	17. 89	1:4. 52

^a The data in this table were taken from the report by Feder (2).

The bone marrow of sample No. 2, which was gelatinous and translucent in character, had the following composition: Moisture 93.13, fat 0.16, and ash 1.5 per cent, as compared with 92 per cent fat and 3.4 per cent moisture in normal bone marrow.

Feder also studied the distribution of nitrogenous compounds in the water extract from a number of samples of flesh. He found that the water extract from those samples having the lower "Feder" numbers (ratio nonfatty organic matter to moisture) contained a higher proportion of nitrogenous compounds that were not precipitated by trichloroacetic acid than did samples of flesh having higher "Feder" numbers. Feder states that in certain cases the flesh of poorly nourished or diseased animals may show higher ratio numbers than 4.0.

TERMS USED IN INVESTIGATION

Before presenting the results of this investigation it seems necessary to define the terms "very thin" and "extremely emaciated" as they will be employed in this paper. "Very thin" cattle may be defined as cattle with a much shrunk musculature and with practically no fat deposits, but with no apparent abnormal condition of flesh, connective tissue, bone marrow, or organs. "Extremely emaciated" cattle, on the other hand, may be defined as cattle in which emaciation has proceeded to such a degree that the flesh, connective tissue, and bone marrow have become distinctly abnormal in appearance. In this paper these terms will be applied to those animals only that are free from evidence of recognized disease.

MATERIAL USED IN INVESTIGATION

The material used in this investigation consisted of (1) hind quarters from extremely emaciated cattle, (2) hind quarters from very thin cattle. The quarters were obtained from meat-packing establishments in Chicago, Ill., and Kansas City, Kans. As soon as it was thoroughly chilled the beef was wrapped in cheesecloth and burlap and forwarded in refrigerator cars to the Biochemic Division, Bureau of Animal Industry, Washington, D. C.

On receipt at the laboratory the quarters were placed in cold storage at a temperature approximating 36° F. Each quarter was weighed, and a record was made of its physical characteristics. As a rule, the femur was sawed in two, so that the marrow could be examined. The muscle tissue was carefully separated from bone and connective tissue, ground twice, and approximately 2 pounds was transferred to a glass jar for analysis. The chemical determinations were begun either the same day that the meat was prepared for analysis or the following morning.

DESCRIPTION OF HIND QUARTERS FROM EXTREMELY EMACIATED CATTLE

In all, 20 hind quarters from a like number of extremely emaciated cattle were examined. It is not worth while to describe each quarter in detail, since all had the same general characteristics, which were as follows:

(1) There was practically complete absence of visible fat, although a slight deposit was found between the muscle bundles in one or two quarters. The

connective tissue about the kidneys, where fat had previously been deposited, was slimy in character.

(2) The marrow in the femur was gelatinous, translucent, and usually semi-fluid in character.

(3) All quarters were extremely emaciated, the musculature being much shrunken, particularly on the loin.

(4) The muscle tissue was soft and flabby, and often rather sticky on the cut surface; as a rule, it was very watery. In some instances the muscles were dark in color, in other cases light, but the bright red characteristic of fat beef was always absent.

(5) As a rule the connective tissue between the muscle bundles was watery and slimy.

The weights of individual quarters ranged from 49 to 124 pounds, the average being 76 pounds. The time which elapsed between the slaughter of the cattle and the receipt of the quarters of beef at the laboratory ranged from 5 to 12 days, the average being $7\frac{1}{2}$ days. The meat was under refrigeration practically all this time.

DESCRIPTION OF HIND QUARTERS FROM VERY THIN CATTLE

Twelve hind quarters from very thin carcasses, corresponding to a like number of cattle, were examined. The weights of the quarters ranged from 47 to 86 pounds, the average being 61 pounds. The time which elapsed between the slaughter of the cattle and the receipt of the beef at the laboratory ranged from 5 to 7 days. Brief descriptions of the several quarters of beef are given in the following:

Samples Nos. 766, 767, 768, 770, 771, and 772 were very similar in appearance, being very thin with a much shrunken and rather flabby musculature. Kidney fat was practically lacking, and fat between the muscle bundles was scant. The marrow in the femur was firm.

In sample No. 999 the musculature was much shrunken, particularly over the loin, and the cut surface of the muscle was lighter in color than normal for fat beef. The connective tissue between the muscle bundles was somewhat watery. There was no fat on the exterior of the round or loin, and only a small quantity about the kidneys and between the muscle bundles. The marrow in the femur was gelatinous in appearance, but of firm consistency.

Sample No. 1000 was similar in appearance to No. 999 except that the connective tissue between the muscle bundles was rather more watery and the marrow in the femur was gelatinous in appearance and soft.

Sample No. 1004 was a thin quarter of beef with only slight deposits of fat between the muscle bundles and with practically no kidney fat. The muscle tissue was firm and of fair color, and the connective tissue was normal in appearance. The marrow in the femur was firm and appeared to contain some fat.

In sample No. 1005 the muscle tissue was rather soft, watery, and light in color. The connective tissue between the muscle bundles was somewhat watery. There was no kidney fat. This quarter of beef was in appreciably poorer condition than No. 1004.

Sample No. 1008 was a thin quarter of beef with no fat deposits. The muscle tissue was rather soft and the connective tissue between the muscle bundles was somewhat watery in character. The bone marrow was reasonably firm.

Sample No. 1010, which was from the carcass of a bull, was of rather better quality than any of the other quarters of beef in this group.

The musculature was fairly well developed and of firm consistency, and the freshly cut surface of the meat was normal in appearance. There was no kidney fat and only a little intramuscular fat. The marrow in the femur was of firm consistency. The connective tissue between the muscle bundles was a trifle watery.

METHODS OF ANALYSIS

For the most part the methods used were those previously described by the authors (6). Purines were determined by the method of Krüger and Schittenhelm (9), creatinine by Folin's method (3), glycogen by Pflüger's method as described by Grube (5), sugar by Hoag and's method (7), and urea by the urease method (8). All determinations were made in duplicate, and the averages of closely agreeing results are reported.

COMPOSITION OF FLESH FROM EXTREMELY EMACIATED CATTLE

At first, in order to ascertain in what important respects, if any, the flesh of this class of cattle differs in composition from that of fat cattle, a rather complete analysis was made of the muscle tissue from each of three hind quarters obtained from a like number of extremely emaciated cattle. These data are reported in Table III, together with analyses of fat cattle obtained in a previous investigation. Several very marked differences in the composition of the lean meat from the two classes of cattle are apparent. The flesh from the extremely emaciated cattle is characterized by a much higher moisture content and by a wider ratio between protein and moisture than the flesh from the fat cattle. On the other hand, the flesh from fat cattle contains an appreciably higher proportion of each of the following constituents: Ash, ether extract, total nitrogen, total protein, total phosphorus, soluble phosphorus, soluble organic phosphorus, and free acid. The percentages of purine and creatinine in the flesh of the extremely emaciated cattle are within normal limits, but the apparent absence of sugar is noteworthy, since good-quality beef usually contains between 0.15 and 0.50 per cent dextrose.

TABLE III.—Composition of flesh from extremely emaciated cattle as compared with that from fat cattle
[Results are expressed in terms of percentages of the fresh material]

Constituent	Extremely emaciated cattle			Fat cattle, average of 5 steers
	No. 689	No. 707	No. 711	
	Per cent	Per cent	Per cent	Per cent
Moisture.....	79.96	79.75	81.39	74.20
Ash.....	.96	1.01	.96	1.07
Ether extract.....	.43	.34	.38	2.71
Total nitrogen.....	3.14	3.14	2.88	3.44
Protein (N×6.25).....	19.63	19.63	18.00	21.50
Ratio, protein to moisture.....	1:4.1	1:4.1	1:4.5	1:3.5
Soluble nitrogen.....	.74	.98	.96	.98
Coagulable nitrogen.....		.59	.62	.55
Amino nitrogen.....	.109	.085	.073	.089
Purine nitrogen.....	.050	.055	.055	
Purine.....	.110	.120	.120	
Creatinine.....	.35	.37	.30	
Dextrose.....	None.	None.		
Total phosphorus.....	.175	.177	.158	.203
Soluble phosphorus.....	.136	.136	.125	.155
Soluble inorganic phosphorus.....	.117	.120	.110	.111
Soluble organic phosphorus.....	.019	.016	.015	.044
Acidity.....	.50	.42	.35	.74

In order to correct for the effects of the different percentages of fat present in the flesh of the two classes of cattle on the content of the other constituents the results of the analyses have been calculated to the fat-free basis, and these data are presented in Table IV. It is apparent that the composition of the fat-free flesh of the extremely emaciated cattle is not materially different from that of the original material, since the fat content of these samples was very low. In the case of the fat cattle, however, the percentage of each of the constituents, except fat, is materially raised when the results are calculated as percentages of the fat-free material, but the same general differences in the composition of the flesh of the two classes of cattle remain.

TABLE IV.—Composition of flesh from extremely emaciated cattle compared with that from fat cattle
[Results are expressed in terms of percentages of the fat-free material]

Constituent	Extremely emaciated cattle			Fat cattle, average of 5 steers
	No. 689	No. 707	No. 711	
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Moisture.....	80.31	80.05	81.70	76.27
Ash.....	.96	1.01	.96	1.10
Total nitrogen.....	3.15	3.15	2.89	3.54
Protein (N×6.25).....	19.69	19.69	18.06	22.09
Soluble nitrogen.....	.74	.98	.96	1.01
Coagulable nitrogen.....59	.62	.57
Amino nitrogen.....	.109	.085	.073	.091
Purine nitrogen.....	.050	.055	.055
Purines.....	.110	.120	.120
Creatinine.....	.35	.37	.30
Total phosphorus.....	.176	.178	.159	.209
Soluble phosphorus.....	.137	.136	.125	.159
Soluble inorganic phosphorus.....	.118	.120	.110	.114
Soluble organic phosphorus.....	.019	.016	.015	.045
Acidity.....	.50	.42	.35	.76

The average moisture content of the three samples of flesh from the extremely emaciated cattle is 80.68 per cent as compared with 76.27 in that from the fat cattle, a difference of 4.41 per cent. The average percentages of ash in the flesh from the two classes of cattle are 0.98 and 1.10 per cent, respectively, and the average percentages of protein are 19.15 and 22.09 per cent. The percentage of total phosphorus is much higher in the flesh from the fat steers than in that from the emaciated cattle, the average percentages being 0.209 and 0.171, respectively. On the other hand, no material differences were found in the total soluble, coagulable, or amino nitrogen in the muscle tissue of the two classes of animals, which apparently indicates that extreme emaciation in cattle is not accompanied by an increase in the solubility of the muscle proteins.

It has been suggested that there might be an accumulation of certain end products of metabolism in the flesh of badly emaciated cattle resulting from the failure of the kidneys to function normally. It was found, however, that the percentages of purines and creatinine in the muscle tissue of this class of cattle were within normal limits.

When the data in Table IV were calculated to the moisture-free basis it was found that, except as to sugar, the flesh of the two classes of animals did not differ materially in composition. This indicates that the differences in composition reported in Table IV are due chiefly to the greater moisture content of the flesh from the emaciated cattle.

In continuing the chemical examination of emaciated carcasses the analytical work was restricted to those constituents which, in the light of the preliminary experiments, appeared to be of significance, viz, moisture, ash, fat, total nitrogen, and sometimes sugar and urea. Twenty hind quarters of beef from a like number of extremely emaciated cattle were examined and the results are reported in Table V.

TABLE V.—*Composition of flesh of extremely emaciated cattle*

[Results are expressed in terms of percentages of the fresh material]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Ether extract	Urea	Glycogen	Dextrose
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
689.....	79.96	0.96	3.14	19.63	1:4.1	0.43			None.
707.....	79.75	1.01	3.14	19.63	1:4.1	.34			None.
711.....	81.39	.96	2.88	18.00	1:4.5	.38			
737.....	79.77	1.04	3.12	19.50	1:4.1	.93			
739.....	82.11	.88	2.77	17.31	1:4.7	.46			
747.....	79.68	.91	2.99	18.69	1:4.3	.71			
748.....	77.38	.96	3.24	20.25	1:3.8	1.13			
749.....	78.87	.99	3.26	20.38	1:3.9	.58			
752.....	79.59	1.07	3.11	19.44	1:4.1	.28			
809.....	80.97	.96	3.02	18.88	1:4.3	.29			
810.....	79.76	.98	3.12	19.50	1:4.1	.33			
811.....	81.24	1.05	2.91	18.19	1:4.5	.38			
900.....	81.20	.92	2.92	18.25	1:4.4	.62			
901.....	80.75	.96	2.90	18.13	1:4.5	.54			
1006.....	79.74	1.01	3.09	19.31	1:4.1	.45	0.012	None.	None.
1009.....	79.75	.94	3.02	18.88	1:4.2	.33	.012	None.	None.
1085.....	80.40	.99	2.96	18.50	1:4.3	.25	.022		None.
1087.....	79.02	1.08	3.14	19.63	1:4.0	.23	.023		None.
1100.....	80.21	1.00	2.85	17.81	1:4.5	.22	.006		0.18
1101.....	80.31	1.03	2.89	18.06	1:4.4	.21			.16
Maximum.....	82.11	1.08	3.26	20.38	1:4.7	1.13			
Minimum.....	77.38	.88	2.77	17.31	1:3.8	.21			
Average.....	80.09	.99	3.02	18.90	1:4.2	.45			

The flesh of the extremely emaciated cattle was again found to be characterized by a high moisture content as compared with that of fat cattle. The percentages of moisture in the 20 samples of flesh reported in Table V range from 77.38 to 82.11, the average being 80.09. Eighteen samples contained in excess of 79 per cent of moisture. By referring to Table III it will be noted that the average moisture content of the flesh from five fat cattle is 74.20 per cent, or when the results are calculated on the fat-free basis the percentage of moisture is 76.27 (Table IV).

The percentage of ash in the muscle tissue from the emaciated cattle ranges from 0.88 to 1.08, the average being 0.99. These figures are to be compared with an average of 1.07 per cent ash in the muscle tissue from fat cattle (Table III) or to 1.10 per cent in the fat-free muscle tissue from the same animals (Table IV).

The protein content of the samples of flesh from the emaciated cattle ranges from 17.31 to 20.38 per cent, the average being 18.90 per cent. The percentage of protein in the flesh from fat cattle is 21.50 (Table III), or 22.09 in the fat-free tissue (Table IV).

The ratio between protein and moisture in the samples of flesh from the emaciated cattle ranges from 1:3.8 to 1:4.7, the average being 1:4.2. Seventeen of the twenty samples show ratios wider

than 1:4.0. By referring to Table III it will be noted that the average ratio between protein and moisture in the flesh from fat cattle is 1:3.5.

The fat content of the flesh from the extremely emaciated cattle is very low, the percentages ranging from 0.21 to 1.13 per cent, the average being 0.45 per cent. Only one sample contains more than 1 per cent of fat. These results are to be compared with 2.71 per cent fat in the flesh from the fat cattle (Table III). It is to be remembered that these data represent analyses of the lean meat which had been trimmed as free from fat as practicable.

Urea was estimated in only five carcasses from extremely emaciated animals. The results obtained indicate considerable variation in the urea content of the flesh from this class of animal. If, on the basis of results reported by Hoagland and Mansfield (8), we take the normal urea content of healthy muscle to be from 0.015 to 0.018 per cent, it is evident from Table V that three of the carcasses contained distinctly subnormal amounts of urea, whereas two contained quantities somewhat above the normal. The low results suggest subnormal metabolic activity; the higher results a slight retention of urea. In general, however, the idea that even extreme emaciation in cattle is characterized by any appreciable retention of urea is not supported by these results.

Although it was to have been expected that the glycogen reserve of extremely emaciated animals would be small, the complete absence of glycogen and dextrose from the flesh of any living animal is scarcely in harmony with accepted views. Yet dextrose was found in but two of the eight samples of emaciated flesh examined for this constituent, and glycogen in neither of two samples. In view of the nature of these results it should be pointed out that they do not necessarily indicate that the flesh was free from carbohydrates at the time of slaughter, for post-mortem destruction of carbohydrates might have occurred. In the case of dextrose, also, one must reckon with the possibility that in the reduction with Fehling's solution the reduced copper oxide might have been held in solution by the substances that do not occur in appreciable quantities in normal meat. These possibilities, of course, require further investigation before any far-reaching conclusions as to the carbohydrate content of badly emaciated flesh can be drawn.

COMPOSITION OF FLESH FROM VERY THIN CATTLE

The quarters of beef that were used in these tests were of very poor quality, and the meat was suitable only for canning or for the manufacture of sausage.

The moisture content of the lean meat from the very thin cattle ranges from 76.81 to 80.49 per cent, the average being 78.84 per cent. Six of the twelve samples contain moisture in excess of 79 per cent. These figures are to be compared with an average moisture content of 80.09 per cent in the flesh from the extremely emaciated cattle, and 74.20 per cent in that from the fat cattle.

The percentage of ash in the flesh from the very thin cattle ranges from 0.93 to 1.10, the average being 1.03 per cent. The average ash content of the lean meat from the extremely emaciated cattle is 0.99 per cent, and that from the fat cattle 1.07 per cent.

The protein content of the lean meat from the very thin cattle ranges from 18.13 to 21.07 per cent, the average being 19.65 per cent. The average percentage of protein in the flesh from the fat cattle is 21.50, and that from the extremely emaciated cattle is 18.90.

The ratio of protein to moisture in the flesh of the very thin cattle ranges from 1:3.6 to 1:4.4, the average being 1:4.0. Four of the twelve samples show ratios wider than 1:4.0, and three samples show the latter ratio. The average ratio of protein to moisture in the lean meat from the extremely emaciated cattle is 1:4.2, and in the flesh from the fat cattle it is 1:3.5.

The fat content of the lean meat from the very thin cattle ranges from 0.36 to 2.02 per cent, the average being 0.75 per cent. Only two samples contain more than 1 per cent fat. These data are to be compared with an average fat content of 2.71 per cent in the lean meat from the fat cattle, and 0.45 per cent in that from the extremely emaciated cattle.

The average urea content of six samples of flesh from very thin cattle was 0.12 per cent, as compared with an average of 0.15 per cent in the lean meat from extremely emaciated carcasses.

Reducing sugar was determined in six samples of lean meat from very thin cattle, but only a trace of sugar was found in one sample.

TABLE VI.—*Composition of flesh of very thin cattle*

[Results are expressed in terms of percentages of the fresh material]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Ether extract	Urea	Dextrose
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
766.....	76.81	1.08	3.37	21.06	1:3.6	1.13		
767.....	77.67	1.08	3.25	20.31	1:3.8	2.02		
768.....	79.34	1.04	3.19	19.94	1:4.0	.39		
770.....	79.96	1.01	2.99	18.69	1:4.3	.48		
771.....	78.37	1.10	3.21	20.06	1:3.9	.48		
772.....	78.52	1.07	3.23	20.19	1:3.9	.42		
999.....	79.41	1.04	3.19	19.94	1:4.0		0.013	Absent.
1000.....	80.49	.97	2.90	18.13	1:4.4	.76	.011	Absent.
1004.....	78.21	1.03	3.16	19.75	1:4.0	.88	.011	Absent.
1005.....	80.11	.96	2.95	18.44	1:4.3	.68	.013	Absent.
1008.....	79.69	.93	3.02	18.88	1:4.2	.59	.010	Absent.
1010.....	77.48	1.01	3.27	20.44	1:3.8	.36		Trace.
Maximum.....	80.49	1.10	3.37	21.06	1:4.4	2.02	.013	
Minimum.....	76.81	.93	2.90	18.13	1:3.6	.36	.010	
Average.....	78.84	1.03	3.14	19.65	1:4.0	.75	.012	

In Table VII are reported the analyses of the samples of flesh from the very thin cattle, expressed in terms of percentages of the fat-free material. With the exception of two samples, Nos. 766 and 767, it will be noted that the composition of the fat-free flesh does not differ materially from that of the fresh material as reported in Table VI.

TABLE VII.—Composition of flesh of very thin cattle
[Results are expressed in terms of percentages of fat-free flesh.]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Urea	Dextrose
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>
766.....	77.69	1.09	3.41	21.31	1:3.6		
767.....	79.27	1.10	3.32	20.75	1:3.8		
768.....	79.65	1.04	3.20	20.00	1:4.0		
770.....	80.34	1.01	3.04	19.00	1:4.3		
771.....	78.75	1.11	3.23	20.19	1:3.9		
772.....	78.85	1.07	3.24	20.25	1:3.9		
999 ^a					1:4.0		
1000.....	81.10	.98	2.92	18.25	1:4.4	0.011	None.
1004.....	78.90	1.04	3.19	19.94	1:4.0	.011	None.
1005.....	80.66	.97	2.97	18.56	1:4.3	.013	None.
1008.....	80.16	.94	3.04	19.00	1:4.2	.010	None.
1010.....	77.76	1.01	3.28	20.50	1:3.8		Trace.
Maximum.....	81.10	1.11	3.41	21.31	1:4.4	.013	
Minimum.....	77.69	.94	2.92	18.25	1:3.6	.010	
Average.....	79.38	1.03	3.17	19.80	1:4.0	.011	

^a Results not calculated to fat-free basis on account of loss of fat determination.

The average composition of the fat-free flesh from each of the three classes of cattle—namely, fat, very thin, and extremely emaciated—are reported in Table VIII.

TABLE VIII.—Average composition of flesh from extremely emaciated, from very thin, and from fat cattle
[Results are expressed in terms of percentages of fat-free material]

Class of cattle	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Extremely emaciated.....	80.45	0.99	3.03	18.94	1:4.2
Very thin.....	79.38	1.03	3.17	19.80	1:4.0
Fat.....	76.27	1.10	3.54	22.09	1:3.5

NOTE.—Data in question were calculated from Table V, which includes all extremely emaciated carcasses examined, rather than from Table IV, which included only the first three.

DISCUSSION OF RESULTS

Emaciation in cattle, except that caused by recognizable disease, must be considered as due to inadequate or faulty nutrition. It is a condition which develops when the destruction of body tissues takes place more rapidly than the processes of repair and growth, the nutritive reserves of the body becoming exhausted and the animal being obliged to utilize the muscle and other tissues in order to support life. Emaciation naturally differs in degree according to the extent to which the animal has used up its own tissues in order to meet its nutritive requirements. From the standpoint of food control it is important to know when this process of disintegration has proceeded to such an extent as to render the flesh of an animal unsuited for human consumption.

The investigation which was conducted by Trowbridge, Moulton, and Haigh (13) has furnished valuable information concerning emaciation in cattle caused by lack of feed. Particularly interest-

ing are the effects of long-continued undernutrition on the weights of the carcasses and organs of steers, as shown in Table I. In addition to these data it is reported that the steer that had been fed the submaintenance ration for the shorter period (6 months) had lost more than two-thirds of its adipose tissue, and the animal fed the same ration for the longer period (11 months) had lost 95 per cent of its fatty tissue. Also, the bone marrow of the latter steer contained practically no fat.

Moulton (11) determined the composition of the flesh of the above-mentioned steers, and as a result he concludes that "inanition or partial starvation does not cause a watery musculature." Although this statement is fully warranted as applying to the two animals in question, it is hardly justified as applying to emaciated cattle in general, as is apparent from the analyses which have been made of the flesh of cattle in advanced stages of emaciation (Tables III and IV), and from the work of Feder (2) (Table II).

From an examination of the data which have been presented herein concerning the composition of the flesh of fat, extremely emaciated, and very thin cattle, it is apparent that the flesh of the extremely emaciated cattle differs very materially from that of the fat cattle, and to a lesser extent from that of the very thin cattle. Although these differences are apparent in the moisture and in the protein content of the flesh from the two classes of animals, they are manifested more clearly and consistently in the protein-moisture ratio.

This ratio has already found use in the detection of water added to sausage, in which connection a ratio of 1: 4.0 is generally recognized as the upper limit for moisture in fresh, sound meat. Thus, as a consequence of investigations carried on from 1909 to 1912 by E. A. Boyer, in charge of the meat-inspection laboratory at Omaha, Nebr., this ratio has been used in the laboratories of the Bureau of Animal Industry since 1913 as a basis for the detection of added water in sausage. Although the result of Boyer's investigation has never been published, reference is made to it in the report of the Chief of the Bureau of Animal Industry for the fiscal year ended June 30, 1913 (10), in which it is stated that the addition of water to sausage may be detected by determining the protein-moisture ratio of the finished product.

The use of the ratio of protein to moisture, or of nonfatty organic matter to moisture, has also been used for some time in Germany as a basis for detecting added water in chopped meat or sausage. Thus Feder (1), in one of his earlier communications, recommended the use of the ratio between the fat-free organic matter and water as a basis for the detection of added water in meat-food products, pointing out that this ratio is fairly constant for fresh meats, and in case of beef or pork sausage meats does not vary appreciably from 1: 4.0.

This early work of Feder has been confirmed by later work by the same author and by that of other German investigators, so that now the ratio of nonfatty organic matter to moisture appears to have found widespread use in Germany as a means of detecting added water in sausage and minced meat. The ratio of 1: 4.0 is generally accepted as the standard for normal meat.

Grossfeld (4) found that the protein content ($N \times 6.25$) did not differ appreciably from that of organic nonfatty material, and so recommended the use of the ratio between protein and moisture

(1:4.0) as a standard for the detection of added water, remarking that the value for protein is more quickly, more simply, and more cheaply obtained than that for fat-free organic matter.

Pannwitz and Harder (12) report the analyses of 102 samples of ground fresh beef, of which only 2 show protein-moisture ratios wider than 1:4.0, and it was found that these contained added water and salt.

Referring to the writers' investigations, it will be noted from Table V that the ratio of protein to moisture in the 20 samples of flesh from extremely emaciated cattle ranges from 1:3.8 to 1:4.7, the average being 1:4.2. Seventeen of the twenty samples show ratios wider than 1:4.0. It may be noted also that the three samples having the narrowest ratios likewise contain the lowest percentages of moisture, and two of the three samples contain the highest percentages of protein.

From Table VII, in which is shown the composition of the flesh from 12 very thin cattle, it will be noted that the ratio of protein to moisture ranges from 1:3.6 to 1:4.4, the average being 1:4.0, the maximum standard set by Feder for normal flesh. Four of the twelve samples of flesh, Nos. 770, 1000, 1005, and 1008, show ratios wider than 1:4.0, namely, 1:4.3, 1:4.4, 1:4.3, and 1:4.2, respectively. It may be noted also that these four samples contain higher percentages of moisture (fat-free basis) than any of the other samples from this group of cattle, each containing in excess of 80 per cent, and that they also contain lower percentages of protein than the other samples.

In describing the several quarters of beef from very thin cattle attention has already been called to the watery condition of the intramuscular connective tissue of samples Nos. 1000, 1005, and 1008. The flesh from each of these quarters of beef had a protein-moisture ratio wider than 1:4.0. Although sample No. 770 had a ratio of 1:4.3, the description of this quarter of beef does not indicate that the flesh or connective tissue appeared to be more watery than that of other quarters of beef having ratios of 1:4.0 or narrower.

As judged by its percentages of protein and moisture and by the ration of protein to moisture, it thus appears that the flesh from four carcasses which had been classed as very thin resembled very closely that of other carcasses that had been classed as extremely emaciated. Whether this similarity indicates an error in the original classification of the carcasses on the basis of physical characteristics, or whether it indicates that the protein-moisture ratio is not an infallible index of the condition of the carcass, is not entirely clear. However, in view of the difficulty experienced in making a rational classification on the basis of physical appearance alone, the writers are inclined to the former alternative. But whichever alternative we accept, it is still evident that in the great majority of cases the protein-moisture ratio can be satisfactorily correlated with the physical condition of the carcass, and it is believed that this ratio may serve a useful purpose in distinguishing mere thinness from extreme emaciation in cattle.

CONCLUSIONS

The results of the investigation reported in this paper appear to justify the following conclusions:

1. The flesh of extremely emaciated cattle is characterized by a relatively high moisture content and by a low content of fat, protein, ash, and probably of sugar.

2. The ratio of protein to moisture in the flesh of extremely emaciated cattle, with but rare exceptions, is wider than 1:4.0, whereas the ratio for the flesh of normal cattle is usually much narrower.

3. It is believed that the ratio between protein and moisture in the flesh of "very thin" or "extremely emaciated" cattle will prove to be of value in classifying such animals for food purposes.

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COMPOSITION OF MARROW OF FRESH AND CURED HAMS ¹

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During a recent conference of meat inspectors of the Bureau of Animal Industry engaged in the supervision of operations of establishments at which Federal meat inspection is maintained, the subject of marrow souring in ham bones was discussed. The question arose whether curing action takes place in the marrow of these bones. Discussion developed the fact that it was not definitely known whether curing took place in the marrow of the bones, and there were sharp differences of opinion among the inspectors on this point. The writers have since examined the marrow of the femur bones from a number of fresh and cured hams in order to determine whether chemical changes had taken place during the curing process.

The femur bones were cut out intact from the hams, all adhering particles of meat were carefully removed, and the bones were washed, in order that the pickle would not come in contact with the marrow. The bones were then cracked, the marrow was removed and the marrow from several hams was combined for each sample, care being taken that no particles of bone were included. The marrow was thoroughly mixed, and the sample was then analyzed.

The methods of the Association of Official Agricultural Chemists were followed in making all determinations. Results of the analyses were as follows:

Marrow from Femur Bones of Fresh Hams

Water	Protein	Fat	Ash	Sodium chloride	Nitrites
8.48	1.6	90.0	0.37	0.14	None.
10.90	1.5	87.7	.19	.14	None.
8.95	1.3	89.9	.17	.12	None.

Marrow from Femur Bones of Hams Cured by Usual Process

13.76	2.5	82.1	1.03	0.60	15 parts per million (as NaNO ₂).
10.58	2.8	85.8	.95	.41	20 parts per million (as NaNO ₂).
9.52	2.3	87.8	.60	.47	55 parts per million (as NaNO ₂).

Marrow from Femur Bones of Hams Cured by Special Process

8.72	1.6	90.0	0.71	0.35	15 parts per million (as NaNO ₂).
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The hams cured by special process were cured in pickle in which sodium nitrite had been used as the color fixative instead of the sodium or potassium nitrate commonly used. The percentage of ash, sodium chloride, and nitrites found were higher in the cured samples than in the fresh. This shows that the curing agents had actually penetrated the marrow of the femur bone.

INACTIVATION OF VITAMIN A BY RANCID FAT¹

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INTRODUCTION

There is, perhaps, little need to be concerned with the characteristics of rancid fat as a human food, since by reason of its impalatability and its unsoundness it is already under the ban of the consumer, producer, and food inspector alike. Nevertheless it has seemed desirable, in the course of studies on rancidity, to carry on a few feeding experiments with rancid fats, partly in order to satisfy a somewhat prevalent curiosity concerning their wholesomeness and partly because of the possible bearing of the results on animal nutrition.

No attempt has been made to evaluate the effect of slightly rancid fats fed in small quantities, as this would probably have entailed a longer study than the importance of the subject seemed to warrant. The chief concern has been rather to discover whether the development of rancidity tends to impair the food value of a fat, and information along this line has been sought by the shortest route possible, viz, by the feeding of intensely rancid fats in comparatively large quantities.

GENERAL PROCEDURE

Three experiments, in which sweet and rancid lard were fed to white rats, were conducted. In each experiment a number of rats selected for uniformity as to age, weight, and general health were divided into two lots, in such manner that each rat in the first lot should have, as far as possible, its counterpart as to sex and parentage in the second lot. One group, used as controls, was fed a ration which was adequate to all intents and purposes and which contained from 16 to 25 per cent of fresh lard, while the other group was given a similar ration containing a corresponding amount of intensely rancid lard. Each rat was confined in a separate cage, which was cleaned at frequent intervals. About twice a week the weight of each rat was charted. In case of the rats receiving the fresh lard, the rations were renewed before any noticeable rancidity had developed in the old ration; and in all cases both the new and the residual rations were weighed when the rations were renewed.

The two lots of fat employed in these experiments were good grades of kettle-rendered lard that had been prepared under observation at a local rendering plant and transported directly to the laboratory in completely filled glass-stoppered bottles. In each case part of the lard, in its original containers, was stored in the dark at 34° F., while the remainder was transferred to a large flask placed before a light window, where for a period of several weeks it was melted and

¹ Received for publication April 30, 1925, issued January, 1926.

aerated daily, care being taken to avoid a temperature greatly in excess of its melting point. When the lard was sufficiently rancid, as indicated by the Kreis test and its odor and taste, the feeding experiment was begun.

EXPERIMENT 1

The rations employed in experiment 1 were made up as follows, the components being intimately mixed in a mortar:

	Per cent
Dry commercial casein-----	14
Cassava starch-----	53
Dried baker's yeast-----	10
Dried egg yolk-----	3
Salt mixture (Drummond and Watson's formula ²)-----	4
Lard (sweet or rancid)-----	16
	<hr/> 100

The sweet fat employed gave a negative Kreis test, and a negative test for peroxides, and contained 0.18 per cent of free fatty acids calculated as oleic acid. The rancid fat gave a positive Kreis test when diluted with 25 parts of pure mineral oil, and an intense test for peroxides, and contained 2.76 per cent of free fatty acids calculated as oleic acid.

Twelve rats were used in this experiment, six of which received the sweet fat and six the rancid. Their ages and weights at the beginning of the experiment, together with their respective sexes and litter numbers, are indicated in Table I.

TABLE I.—Description of rats used in experiment 1

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			<i>Days</i>	<i>Grams</i>					<i>Days</i>	<i>Grams</i>	
1	Rancid---	Female---	32	45	69-C	7	Sweet---	Male---	32	41	69-C
2	do-----	Male-----	32	46	69-C	8	do-----	Female---	33	39	69-D
3	do-----	Female---	32	48	69-C	9	do-----	Male-----	33	40	69-D
4	do-----	do-----	32	48	69-C	10	do-----	do-----	34	40	69-B
5	do-----	Male-----	30	52	70-B	11	do-----	Female---	30	50	70-B
6	do-----	do-----	30	49	70-D	12	do-----	do-----	30	40	70-D

During the earlier stages of the experiment the rats receiving the rancid lard seemed to suffer from a mild diarrhea, indicative of intestinal irritation, but this symptom gradually subsided as the experiment progressed. In general, also, as is shown by the growth curves in Figure 1 and by the record of total food consumption in Table II, the rats of the rancid series consumed less ration and gained less weight than the corresponding controls. Finally, on the eighty-eighth day of the experiment rat No. 3 developed ophthalmia, and on the same day the experiment was terminated.

At the end of the experiment a blood count was made on rat No. 5, which was in the poorest condition of all the rats receiving the rancid lard, and on rat No. 10, which received the sweet lard. No essential difference was noted in the number of red cells in the blood

² DRUMMOND, J. C., and WATSON, A. F. THE TESTING OF FOODSTUFFS FOR VITAMINS. Analyst 47: 237. 1922.

of the two animals, the count being 8,825,000 per cubic millimeter for rat No. 5 and 8,825,000 to 9,025,000 per cubic millimeter for rat No. 10.

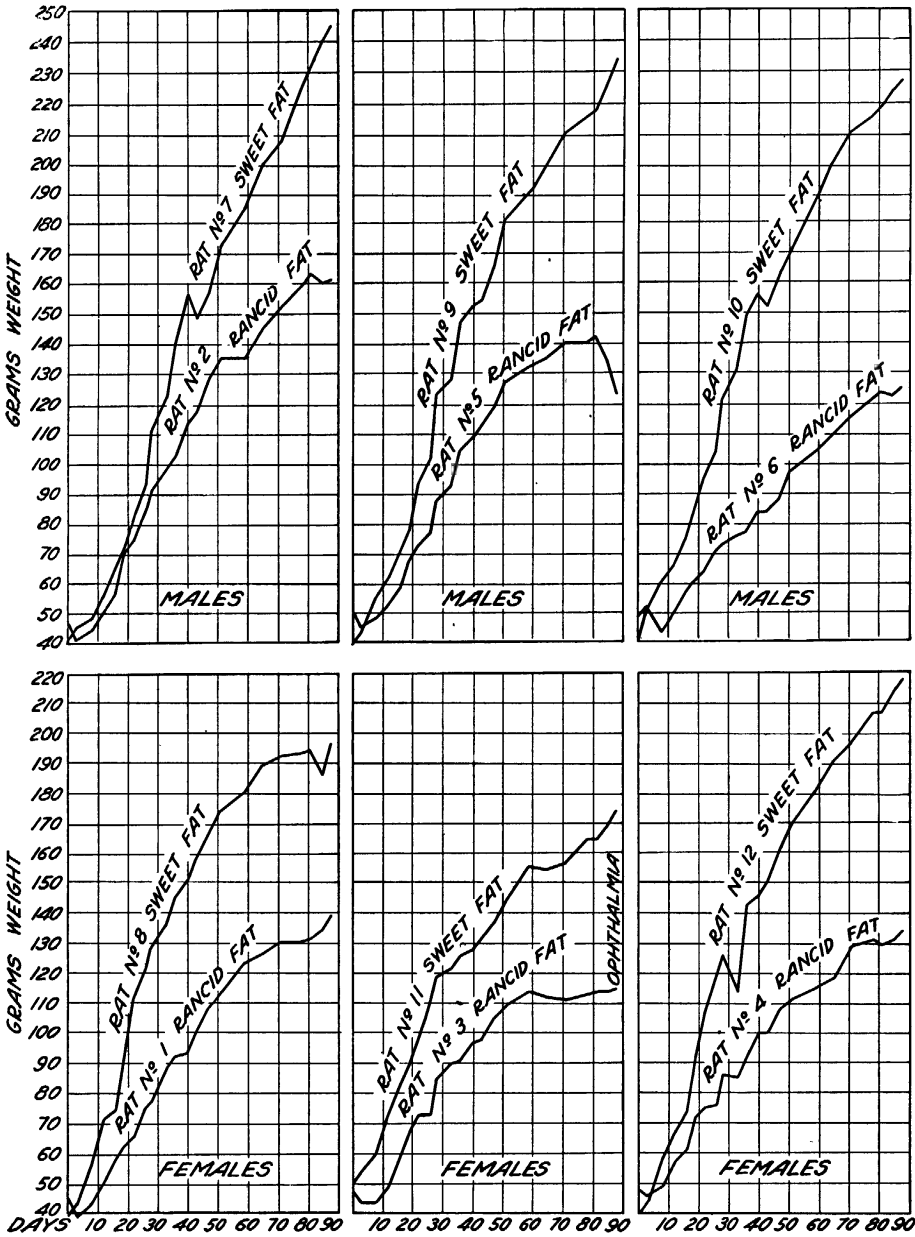


FIG. 1.—Growth curves of rats in experiment 1

Post-mortem examinations of rats Nos. 5 and 6 of the rancid series and of rats Nos. 7 and 10 of the control group yielded the following results:

Rat No. 6.—Practically no mesenteric or kidney fat. No noticeable congestion of mucous membrane of stomach or intestines. Slight congestion of mesenteric blood vessels. Mucous membrane of alimentary tract pale and unhealthy in appearance as compared with that of the controls. The same was true as to tissues in general. The general picture was that of undernutrition.

Rat No. 5.—Similar in condition to rat No. 6, except that there was even less visible fat and that the tissues appeared to be in a poorer state of nutrition. No congestion apparent.

Rat No. 7.—Well nourished, with plenty of fat. Viscera and muscles larger, firmer, and apparently more healthy than those of rats Nos. 5 and 6.
Rat No. 10.—Similar in condition to rat No. 7.

TABLE II.—Food-consumption data for experiment 1, covering first 73 days

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption	Total gain
			Grams	Grams	Grams
Rancid.....	2	Male.....	592	0.0886	108
Do.....	5	do.....	538	.0797	89
Do.....	6	do.....	476	.0846	69
Average for males.....			535	.0843	89
Rancid.....	1	Female.....	508	.0827	85
Do.....	3	do.....	505	.0832	64
Do.....	4	do.....	539	.0857	82
Average for females.....			517	.0839	77
Average for males and females.....			526	.0841	83
Sweet.....	7	Male.....	709	.0854	171
Do.....	9	do.....	679	.0802	172
Do.....	10	do.....	699	.0835	172
Average for males.....			696	.0830	172
Sweet.....	8	Female.....	657	.0777	153
Do.....	11	do.....	594	.0768	109
Do.....	12	do.....	677	.0799	160
Average for females.....			643	.0781	141
Average for males and females.....			669	.0806	156

By interpolation of the growth curves and the records of food consumption, the weight of food consumed per day, per gram of weight of the given rat, was calculated for daily intervals. The average of the values thus obtained throughout the period covered by this table has been called the "coefficient of food consumption" for the rat in question.

In the light of these observations it would appear that rancid lard is not toxic to white rats, and that the subnormal growth of rats Nos. 1 to 6 must be attributed to undernutrition or to vitamin deficiency. Since the rancid ration may have been unpalatable, one immediately suspects that either or both of these conditions might have originated in a self-imposed curtailment of food consumption, and hence of vitamin-A intake, by the rats of the rancid series, under which circumstances even the development of ophthalmia in rat No. 3 would signify nothing as to the adequacy of the ration. But this suspicion is not supported by the food-consumption data as presented in Table II, for the rats of the rancid series consumed an even larger average weight of ration per day and per gram of body weight than the control rats receiving the sweet lard. Apparently, therefore, gross food consumption was limited by growth, not growth by the rate of food consumption; and the cause of this limitation of growth must be sought in the inferior quality of the ration containing the rancid fat as compared with the control ration.

Although the experiment may not be conclusive as to the nature of this inferiority, the development of ophthalmia in rat No. 3 suggests that it consisted in a deficiency of vitamin A, while the similarity of the two rations in other respects would indicate that the inferiority was caused by the presence of the rancid lard. In view of the known susceptibility of vitamin A to oxidation, and of the presence of organic

peroxides in rancid fat, and of the intimate admixture of the rancid lard with the ration fed to rats Nos. 1 to 6, one is inclined to suspect that the rancid lard effected a partial destruction of the vitamin A that was originally added to this ration.

EXPERIMENT 2

In experiment 2 vitamin A was fed separately from the main ration containing the lard, and was thus protected from any injurious contact with rancid fat. The supplemental ration containing the vitamin A was composed of 36 per cent of dried egg yolk and 64 per cent of starch, and was fed to all rats equally in daily portions ranging from 0.3 gram at the beginning of the experiment to 0.8 gram at the end. In this manner sufficient vitamin A was provided to cover the requirements of the most thrifty of the experimental animals.

The main portion of the ration, fed ad libitum, was made up as follows:

	Per cent
Dry commercial casein.....	13. 66
Cassava starch.....	47. 45
Dried baker's yeast.....	9. 76
Salt mixture ³	3. 90
Lard (sweet or rancid).....	25. 23
	<hr/> 100. 00

Naturally, each rat consumed a different amount of this ration, so that the quantitative composition of the total food derived from both rations varied from day to day and from rat to rat. The relative amount of the several food materials consumed by each group of rats as a whole, for the entire period of the experiment, however, was as follows:

	Per cent		Per cent
Sweet lard.....	22. 13	Rancid lard.....	20. 35
Casein.....	12. 00	Casein.....	11. 00
Starch.....	49. 47	Starch.....	50. 65
Dried yeast.....	8. 56	Dried yeast.....	7. 87
Salt mixture.....	3. 42	Salt mixture.....	3. 15
Dried egg yolk.....	4. 42	Dried egg yolk.....	6. 98
	<hr/> 100. 00		<hr/> 100. 00

The lard used in this experiment, though from a different lot, was of the same type and quality and was obtained and prepared in the same manner as that used in experiment 1. At the beginning of the experiment the sweet lard contained 0.32 per cent of free fatty acids calculated as oleic acid, and gave a negative Kreis test and a negative test for peroxides. The rancid lard, on the other hand, contained 0.48 per cent of free fatty acids calculated as oleic acid, and gave a positive Kreis test when diluted with 17 parts of pure mineral oil, and an intense test for peroxide.

Eight rats were used in this experiment, the pertinent data concerning which are recorded in Table III.

³ DRUMMOND, J. C., and WATSON, A. F. Op. cit.

TABLE III.—Description of rats used in experiment 2

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			Days	Grams					Days	Grams	
13	Sweet	Male	25	48	112-B	17	Rancid	Male	25	46	112-B
14	do	Female	25	38	112-B	18	do	Female	25	49	112-B
15	do	Male	25	38	113-A	19	do	Male	25	37	113-A
16	do	Female	25	39	113-A	20	do	Female	^a 26	37	113-A

^a Rat No. 20 was started one day later than the others.

Contrasted with the conspicuous greed of the rancid group for the vitamin-A supplement was the comparative indifference toward it of rat No. 14 and in smaller degree of rats Nos. 13 and 16 of the control group. All the rats in the rancid group, as well as rat No. 15 in the

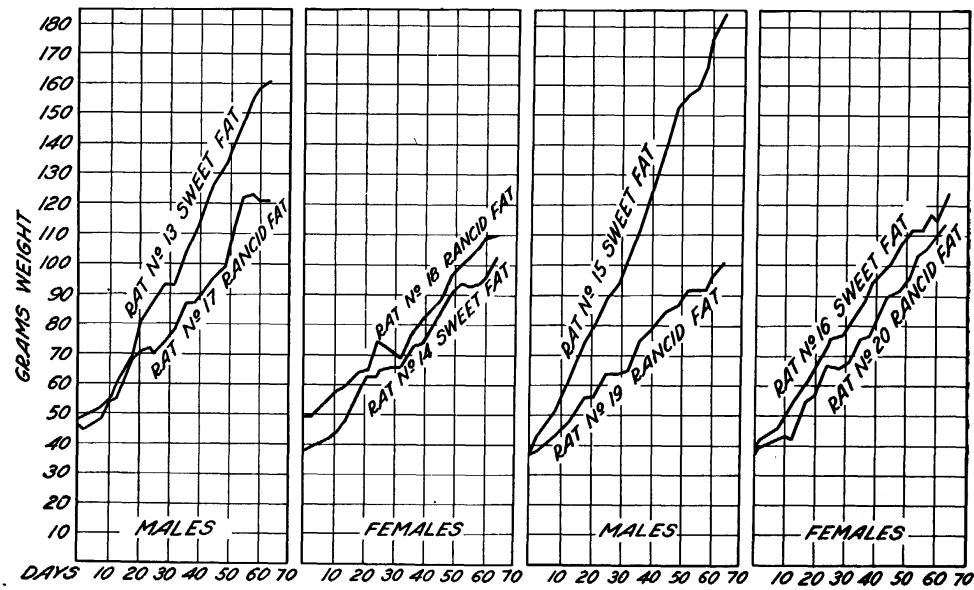


FIG. 2.—Growth curves of rats in experiment 2

control group, regularly consumed their daily supplements within a five-minute period, while rat No. 14 scarcely touched the supplemental ration during the first week of the experiment, and consumed it with but little interest and for the most part incompletely during the remainder of the experiment. Rats Nos. 13 and 16, on the other hand, while they ate the supplemental ration incompletely at the beginning of the experiment, consumed it completely and with an increasing show of interest in the later stages of the experiment. Throughout the experiment all the rats remained healthy and active and free from ophthalmia or other evidence of deficiency disease.

The growth curves for this experiment are presented in Figure 2, and the food-consumption data in Table IV. Evidently no appreciable limitation in the growth of the female rats in the rancid series was occasioned by the fact that they consumed slightly less ration per day per gram of body weight than the females in the control series. Presumably, therefore, diminished food consumption was not the limiting factor in the growth of the male rats of the

rancid series, since the rate of food consumption of the two series was more nearly equal in the case of the males than in the case of the females.

TABLE IV.—*Food-consumption data for experiment 2, covering first 60 days*

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption ^a	Total gain
			Grams	Gram	Grams
Sweet.....	13	Male.....	406	0.0705	110
Do.....	15	do.....	342	.0558	138
Average for males.....			374	.0631	124
Sweet.....	14	Female.....	280	.0691	58
Do.....	16	do.....	274	.0573	76
Average for females.....			277	.0632	67
Average for males and females.....			325	.0632	95
Rancid.....	17	Male.....	300	.0631	75
Do.....	19	do.....	232	.0578	59
Average for males.....			266	.0604	67
Rancid.....	18	Female.....	290	.0665	60
Do.....	20	do.....	209	.0508	72
Average for females.....			249	.0586	66
Average for males and females.....			258	.0595	66

^a See footnote to Table II.

Yet it is somewhat remarkable that, whereas an approximate equivalence in rate of growth should have obtained between the two groups of females, the males of the control series should have grown so much more rapidly than the corresponding males receiving the rancid lard. In view of the fact that the latter rats received fully as much vitamin A as the former, it is difficult to understand the reason for their subnormal growth.

One might, of course, postulate that animal appetite is largely determined by nutritive requirements, and that the greater greed with which the rats of the rancid series consumed their vitamin-A supplements was conditioned by their greater need for vitamin A as compared with the control rats. One might further explain this increased requirement by supposing that the rancid fat, after its ingestion, effected a partial destruction of the vitamin A of the supplemental ration or even of the tissues. It would then be conceivable that the vitamin A actually supplied in this experiment was sufficient to cover the thus increased requirements of the female rats of the rancid series, but was somewhat insufficient to provide for the more rapid growth of which the male rats of the rancid series should have been capable.

But as such reasoning involves a number of rather improbable assumptions, and is highly speculative at best, we may well limit ourselves to observing that in this experiment, where the vitamin A was fed separately, the rancid lard, generally speaking, seemed to affect the growth and the general health of the rats receiving it less detrimentally than was the case in experiment 1, where the rancid lard came into intimate and prolonged contact with the vitamin A.

EXPERIMENT 3

Experiment 3 was a counterpart of experiment 1, except that the lard employed was the same as that used in experiment 2, and that only 8 rats were used instead of 12. The composition of the ration has already been given in connection with experiment 1, and the samples of rancid and sweet lard have been described in connection with experiment 2. The pertinent data concerning the experimental animals at the beginning of the experiment are recorded in Table V.

TABLE V.—Description of rats used in experiment 3

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			Days	Grams					Days	Grams	
21	Sweet	Male	27	42	115-A	25	Rancid	Male	27	41	115-A
22	do	Female	27	42	115-A	26	do	Female	28	42	115-B
23	do	do	28	39	115-B	27	do	do	28	43	115-B
24	do	Male	27	39	115-A	28	do	Male	27	44	115-A

Throughout experiment 3 the rats receiving the rancid fat consumed less food and gained less weight than the corresponding controls. They were also less active and robust, and without exception devel-

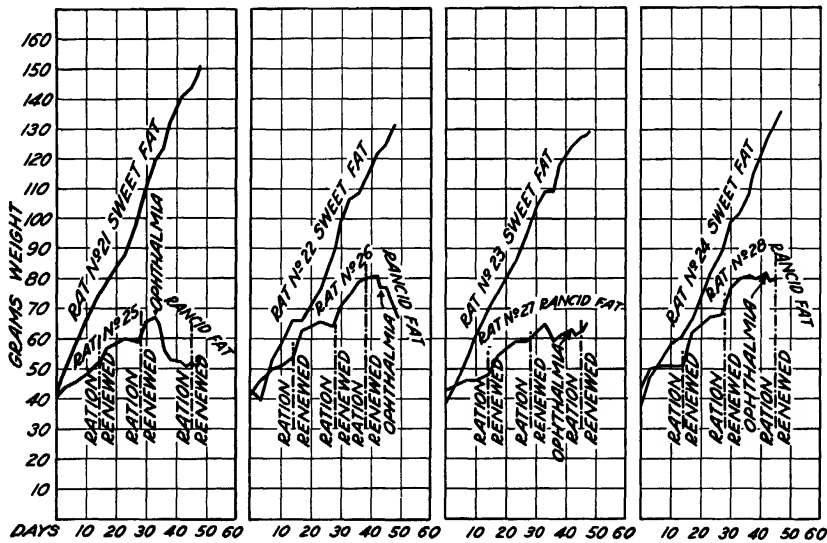


FIG. 3.—Growth curves of rats in experiment 3

oped pronounced cases of ophthalmia before the end of the experiment. In case of rat No. 25 of the rancid series, moreover, the hair remained scant and thin, the pink skin being clearly visible over the rump throughout the experiment. On the other hand, the rats of the rancid series manifested no definite symptoms of intestinal irritation as did the corresponding rats in experiment 1.

The smaller growth made by the rats in the rancid series as compared with the control rats is clearly shown by the growth curves in Figure 3. In case of the rats receiving the rancid fat, the subnormal growth, the development of ophthalmia, and the ultimate decrease in weight of rats Nos. 25, 26, and 28 bear clear testimony of a deficiency of vitamin A. From the data on food consumption presented

in Table VI, however, it is obvious that the rats of the rancid series consumed considerably less ration per day per gram of body weight than the corresponding controls; and we are left in doubt as to whether the deficiency was caused by a disappearance of vitamin A from the ration or by the limited food consumption. The average food consumption of the rats of the rancid series per day per gram of body weight, however, was 80 per cent of that of the control rats; and in view of the fact that the amount of egg yolk originally added to the ration was considerably in excess of that required for optimal growth of normal rats, we are inclined to believe that in spite of their limited food consumption the rats of the rancid series received enough egg yolk to have protected them from ophthalmia, if not to insure normal growth, had the vitamin A retained its potency. Therefore, while limited food consumption may have been, and probably was, a limiting factor in the growth of these rats, the evidence seems also to point to a loss of vitamin A from the ration containing the rancid lard.

TABLE VI.—Food-consumption data for experiment 3, covering third to forty-eighth day, inclusive

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption ^a	Total gain
			Grams	Grams	Grams
Sweet.....	21	Male.....	371	0.0874	101
Do.....	24	do.....	324	.0856	94
Average for males.....			347	.0865	97
Sweet.....	22	Female.....	378	.1038	93
Do.....	23	do.....	320	.0806	85
Average for females.....			349	.0922	89
Average for males and females.....			348	.0893	93
Rancid.....	25	Male.....	180	.0740	10
Do.....	28	do.....	204	.0676	21
Average for males.....			192	.0708	15
Rancid.....	26	Female.....	223	.0762	21
Do.....	27	do.....	172	.0681	21
Average for females.....			197	.0721	21
Average for males and females.....			194	.0715	18

^a See footnote to Table II.

This idea is further supported by the periodic nature of the growth curves of the rats in the rancid series. These curves have been marked at points corresponding to the introduction of freshly mixed ration, and it will be observed that the periods of accelerated growth invariably follow closely upon the renewal of the ration. While the records of food consumption are not sufficiently detailed to show whether or not the accelerated growth was accompanied by increased food consumption, it is clear that the ration containing the rancid lard, unlike the ration containing the sweet lard, deteriorated with increasing age; and the most plausible explanation of this fact would seem to be that the vitamin A of the ration was gradually destroyed by contact with the rancid fat.

This experiment, therefore, seems to confirm the inference already drawn from experiment 1, viz, that rancid fat apparently destroys

some of the vitamin A of food products with which it may be intimately mixed. It further suggests that the destruction of vitamin A, under the conditions here obtaining, proceeds gradually over a period of several days.

SUMMARY

Three experiments with rats bearing upon the characteristics of rancid lard as a food are reported. The lard used was more intensely rancid than would anywhere have been tolerated for human consumption, and probably more rancid than would be acceptable for animal feeding. It was also employed in larger proportions than those in which fat is generally used in the rations of man or beast. The results may be summarized as follows:

Under the conditions of these experiments, rancid lard, though clearly inferior to sweet lard as a ration component, did not seem to be actually toxic to the white rats receiving it.

The inferiority of rancid lard as a food product appeared to be due to its ability to destroy the vitamin A of the rations with which it was admixed. This destruction appeared to occur gradually over a period of several days, and was presumably due to the oxidation of the vitamin A by the organic peroxides of the rancid lard.

When the vitamin A was fed separately from the lard, the males receiving the rancid lard seemed to require more vitamin A than did males receiving a corresponding ration containing sweet lard.

THE LEAF-TISSUE FLUIDS OF EGYPTIAN COTTONS¹

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INTRODUCTION

In 1921 a series of determinations of the physicochemical properties of the leaf-tissue fluids of Egyptian and Upland cottons and of their F_1 hybrid (3)² showed that, when grown under irrigation at Sacaton, in the Gila River Valley of southern Arizona, the leaf-tissue fluids of Pima Egyptian cotton are characterized by higher osmotic concentration, higher specific electrical conductivity, and higher hydrogen-ion concentration than those of Meade or Acala Upland cotton (3).

In subsequent studies it has been shown that the differentiation is also clearly marked in chloride (4) and in sulphate (7) content, and that the absorption of these two ions is differential (5).

The question will naturally arise, however, as to whether the sap properties found for Pima may be considered wholly typical of the Egyptian type in this regard. Kearney has shown (8, 9, 10, 11) that the Pima variety has a long history as an American-grown cotton. It seems quite possible that the differences between its tissue-fluid properties and those of the upland varieties with which it was compared may have arisen during this period in America.

The plausibility of this suggestion is emphasized by Balls' (1) conclusion that in Egypt the different strains of cotton differ in the salinity of their tissue fluids.

One purpose of the present investigation has been to determine whether varieties of Egyptian cotton other than Pima differ from Acala, Meade, and Lone Star Upland cotton in their tissue-fluid properties, or whether this difference is characteristic only of the American Egyptian variety, Pima.

MATERIALS AND METHODS

In 1922 plantings of seeds of five Egyptian varieties imported in that year from Egypt were made for comparison with Pima Egyptian and Meade upland cotton from American-grown seed. In 1924 the new

¹ Received for publication May 28, 1925; issued January, 1926.

² Reference is made by number (italic) to "Literature cited," p. 1033.

varieties were grown from seed which had been rendered more uniform by self-fertilization of plants selected as true to type. Three Upland varieties—Meade, Acala, and Lone Star—as well as Sea-Island cotton—were introduced in the comparison.

The exact cultural details need not be discussed here. The plantings were made in a manner similar to those adopted in earlier experiments. This involves the distribution of all of the varieties over the field in short subrows or subplots. Samples of mature leaf tissue were taken from all of the varieties of a subplot at the same time.

The leaf-tissue fluids were extracted and the physicochemical constants determined in a manner described in an earlier paper (3).

PRESENTATION AND ANALYSIS OF DATA

In the presentation of the results only the averages are given. Probable errors have been omitted for the following reasons: (a) The number of determinations on any one variety is small, and this renders the interpretation of the probable errors difficult under any conditions, particularly under the conditions of the present experiment; (b) the chief use of the probable errors of the means would have been in connection with the determination of the probable errors of the differences between any two varieties.

Such probable errors can be determined only if the correlation between the constants for the two varieties under comparison be known. The determination of such correlations on the basis of as large a number of varieties and as wide a spacing of varieties as are found in these experiments presents considerable difficulty.³

In the determination of the averages a somewhat different method has proved to be desirable for the two experiments.

While the plot utilized in the experiment of 1922 showed great irregularities of soil conditions, which were strikingly evident in the characteristics of the plants, a fairly uniform stand was obtained for the whole plot.

In the experiment made in 1924 the southern portion of the experimental plot carried a fairly uniform stand, but toward the northern end of the field many sections produced no plants at all. These differences in the salinity and texture of the soil, to which the diversities in stand are due, doubtless play an important part in determining the physicochemical properties of the plant-tissue fluids. It has seemed desirable, therefore, to prepare two sets of averages for the determinations made in 1924, the first comprising the southern end of the field, where materials could be obtained for all of the varieties, and the second including the northern end of the field, the stand there being not as good as that on the southern end. These two averages, with indications of the number of determinations on which these are based, are given as "partial" and "whole" series in the tables.

³ It might seem that these difficulties should have been overcome by a different arrangement of the plantings. This, however, was impossible with the number of varieties involved in the present experiments without so reducing the number of plants per subplot that the determinations would have been based on tissues from one or but a very few individuals. In the organization of this experiment it seemed best, all things considered, to increase the size of the individual cultures, even though this resulted in the separation of the groups of plants to be compared.

The average values of osmotic concentration, as expressed in terms of freezing-point depression (Δ) in the various series of determinations made in the two years, are set forth in Table I. In the experiment of 1922, in both the first and the second series of determinations, all six of the Egyptian cottons show a greater freezing-point depression than the single variety (Meade) of Upland cotton.

TABLE I.—Comparison of osmotic concentration in terms of freezing-point depression (Δ) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924 (*E*₁–*E*₅ are field key letters)

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, <i>E</i> ₁	8	1.275	8	1.285	8	1.233	8	1.166	12	1.243	14	1.230
Zagora, <i>E</i> ₂	8	1.298	8	1.291	8	1.238	8	1.134	12	1.240	14	1.215
Sakel, <i>E</i> ₃	8	1.345	8	1.378	8	1.371	8	1.374	12	1.379	15	1.444
Pellon, <i>E</i> ₄	8	1.338	8	1.335	8	1.309	8	1.274	12	1.349	13	1.314
Assili, <i>E</i> ₅	8	1.274	8	1.396	8	1.311	8	1.314	12	1.368	15	1.430
Pima.....	8	1.380	8	1.417	8	1.267	8	1.300	11	1.261	13	1.353
Sea island.....					8	1.151	8	1.134	8	1.151	11	1.152
Upland:												
Acala.....					8	1.112	8	1.061	8	1.112	11	1.078
Meade.....	8	1.251	8	1.247	8	1.147	8	1.086	8	1.147	11	1.146
Lone Star.....					7	1.106	8	1.037	7	1.106	11	1.102

In the series of determinations carried out in 1924 the averages based on the partial series and the entire series of determinations must be considered separately. In both the first and second partial series the freezing-point depression is higher in each of the six Egyptian varieties than in any one of the three different Upland varieties (Acala, Meade, and Lone Star).

In only one instance is the freezing-point depression of an Egyptian variety as low as that of Sea-Island cotton.

In both the first and the second whole series the averages of each of the six Egyptian varieties are numerically higher than those for the Upland varieties or for the Sea-Island cotton.

While the Egyptian varieties differ to some extent among themselves, taken as a class they are all characterized by distinctly higher osmotic concentration than the Upland varieties with which they are compared. These results show that higher osmotic concentration is not a peculiarity of the American Egyptian variety, Pima, but that it is characteristic of Egyptian varieties in general.

The experiments are not sufficiently extensive to justify final conclusions concerning the relative values of osmotic concentration in the Egyptian varieties themselves. It appears, however, that Ashmuni and Zagora have lower osmotic concentrations than the other four types, although in the first series of determinations Assili has about the same average freezing-point depression as the two just mentioned.

Turning now to the values of specific electrical conductivity as expressed in reciprocal ohms, we have the constants set forth in Table II. In all 12 comparisons which may be based upon the series of determinations made in 1922, the value of κ for the Egyptian type is higher than that for the single variety of the Upland type. In 1924 the same result is found for the comparison between the six Egyptian and the three upland types. Thus there can be no question that while the Egyptian varieties differ to some extent among themselves, they are, so far as investigated, characterized by higher specific electrical conductivity than the Upland types which have been grown under similar conditions in the investigations which have hitherto been possible.

TABLE II.—Comparison of specific electrical conductivity (κ) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, E ₁ -----	8	0.03282	8	0.03212	8	0.02949	8	0.02871	12	0.02936	14	0.02960
Zagora, E ₂ -----	8	.03255	8	.03170	8	.02977	8	.02776	12	.02970	14	.02878
Sakel, E ₃ -----	8	.03364	8	.03319	8	.03085	8	.03170	12	.03139	15	.03226
Pelion, E ₄ -----	8	.03463	8	.03307	8	.03149	8	.03135	12	.03223	13	.03231
Assili, E ₅ -----	8	.03288	8	.03199	8	.03000	8	.03070	12	.03109	15	.03208
Pima-----	8	.03449	8	.03339	8	.02948	8	.02991	11	.02878	13	.03034
Sea island-----					8	.02647	8	.02750	8	.02647	11	.02782
Upland:												
Acala-----					7	.02601	8	.02445	7	.02601	11	.02562
Meade-----	8	.03163	8	.03066	8	.02734	8	.02539	8	.02734	11	.02619
Lone Star-----					8	.02511	8	.02431	8	.02511	11	.02417

In both the first and second series of determinations of 1924, as epitomized in the averages for both the partial and the entire series, specific electrical conductivity for the Sea-Island cotton is lower than that of any of the Egyptian varieties. It may be higher or lower than that of the associated Upland cultures.

We now consider the ratio of specific electrical conductivity to freezing-point depression, κ/Δ . The averages are given in Table III. For the first series of determinations the average ratio for the six different Egyptian varieties is identical with that for the Upland type in so far as can be determined by the examination of averages not provided with probable errors. This result is in accord with the findings of an earlier investigation (3) on Pima Egyptian and Meade and Acala Upland cotton, in which the conclusion was drawn that there is no certain differentiation of the two types with respect to the ratio κ/Δ .

We now have to consider the concentration of two anions, for which absorption has been shown to be differential (5).

The results of an earlier investigation (4) have shown conclusively that Pima Egyptian cotton differs from Meade, Acala, and Lone Star Upland cotton in the chloride content of its leaf-tissue fluids.

Apparently Egyptian cotton has a greater capacity for the accumulation of chlorides with the march of the season (6) than has Upland cotton.

TABLE III.—Comparison of the ratio of specific electrical conductivity (κ) to freezing-point depression (Δ) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni.....	8	0.02578	8	0.02506	8	0.02397	8	0.02461	12	0.02366	14	0.02421
Zagora.....	8	.02520	8	.02470	8	.02401	8	.02447	12	.02394	14	.02389
Sakel.....	8	.02512	8	.02426	8	.02254	8	.02311	12	.02278	15	.02250
Pelion.....	8	.02596	8	.02490	8	.02406	8	.02461	12	.02392	13	.02460
Assili.....	8	.02596	8	.02486	8	.02291	8	.02336	12	.02278	15	.02253
Pima.....	8	.02510	8	.02375	8	.02329	8	.02301	11	.02282	13	.02254
Sea island.....					8	.02304	8	.02425	8	.02304	11	.02417
Upland:												
Acala.....					7	.02348	8	.02360	7	.02348	11	.02334
Meade.....	8	.02537	8	.02473	8	.02390	8	.02340	8	.02390	11	.02296
Lone Star.....					8	.02261	8	.02291	8	.02261	11	.02246

The question naturally arises as to whether the higher chloride content is peculiar to the Pima variety of Egyptian cotton, which has developed in the Southwest from a long series of ancestors which had been grown under the frequently saline conditions of this region, or whether it is a characteristic common to Egyptian cottons in general. The results for a series of analyses carried out by a method proposed by Lawrence and Harris (12) appear in Table IV.

TABLE IV.—Comparison of the chloride content (in terms of grams of Cl per liter) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924

Variety	Determinations made in 1922				Determinations made in 1924							
	First series Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, E ₁	7	6.51	8	7.04	7	3.54	8	3.98	11	3.48	14	3.95
Zagora, E ₂	8	6.14	8	6.82	8	3.56	8	3.66	11	3.56	14	4.04
Sakel, E ₃	7	6.07	8	6.50	8	3.50	8	3.82	11	3.60	13	3.94
Pelion, E ₄	7	6.86	8	6.95	8	4.12	7	4.73	12	4.30	11	4.82
Assili, E ₅	8	6.21	8	6.59	7	3.16	7	4.20	11	3.79	13	4.50
Pima.....	7	7.44	8	7.89	7	3.02	8	3.59	10	3.05	13	3.53
Sea island.....					7	2.21	7	2.09	7	2.21	10	2.12
Upland:												
Acala.....					7	2.35	8	2.79	7	2.35	10	2.65
Meade.....	8	4.61	8	4.61	8	1.88	8	2.32	8	1.88	11	2.33
Lone Star.....					7	1.45	7	1.56	7	1.45	11	1.58

The averages for the determinations made in 1922 ⁴ show that all six of the Egyptian varieties have a higher chloride content than the Meade Upland cotton grown as a control, and that in both the first and second series of determinations the chloride content of the Pima Egyptian plants is slightly higher than that of any other Egyptian variety.

Because of certain analytical difficulties, the determinations based on the plants grown in 1924 must be regarded as only approximate. They indicate clearly, however, that all of the Egyptian varieties have a higher chloride content than either Sea-Island or the three Upland varieties included in this experiment.

In another place the writers have shown (7) that the sulphate content of the leaf-tissue of Pima Egyptian cotton is lower than that of the Meade or Lone Star varieties of upland cotton. Determinations on the sulphate content of other Egyptian varieties, made by the method of Gortner and Hoffman (2), are available for the experiment of 1924 only.⁵ The determinations must be regarded as more or less approximate. They show clearly, however, that the sulphate content of the Upland varieties is higher than that of any of the Egyptian varieties considered, and higher than that of Sea-Island cotton.

TABLE V.—Comparison of the sulphate content (in terms of grams of SO_4 per li'er) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1924

Variety	Determinations made in 1924							
	First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:								
Ashmuni, E ₁	8	10.14	8	8.58	12	10.44	13	8.93
Zagora, E ₂	8	9.92	6	10.14	12	10.02	12	10.26
Sakel, E ₃	8	10.15	8	10.01	12	10.41	14	10.19
Pellion, E ₄	8	9.91	7	9.33	12	10.09	11	9.28
Assili, E ₅	8	10.44	7	9.64	12	10.35	10	9.81
Pima.....	8	10.34	8	9.32	10	10.29	13	9.70
Sea-Island.....	8	9.95	7	10.08	8	9.95	10	10.42
Upland:								
Acala.....	7	11.61	8	11.08	8	11.61	11	12.00
Meade.....	8	11.89	8	12.04	8	11.89	11	12.80
Lone Star.....	8	13.00	7	13.38	7	13.00	10	13.73

SUMMARY

The purpose of the present study has been to determine whether Pima Egyptian cotton, a variety of American origin, is unique in that it is different from at least some of the Upland varieties in the physico-chemical properties of the leaf tissue fluids as shown in earlier investigations (3, 4, 5, 6, 7), or whether all of the varieties of the Egyptian type differ from those of the Upland type of cotton.

The constants here considered represent, in addition to Pima, five Egyptian varieties grown from seed imported from Egypt in 1922. These are Ashmuni, Zagora, Sakel, Pellion, and Assili.

⁴ The determinations of the chloride content of the samples of this series were made by Dr. and Mrs. John V. Lawrence.

⁵ The analytical work on this series has been done by Clara T. Hoffman.

The results show that while the Egyptian varieties apparently differ among themselves, all of the six varieties here considered have a higher osmotic concentration and specific electrical conductivity than the upland varieties (Acala, Meade, and Lone Star) with which they have been compared. The two types apparently do not differ in the ratio of specific electrical conductivity to freezing-point depression.

All of the Egyptian forms considered have a higher chloride content and a lower sulphate content than the upland types. It may be recalled in this connection that Balls (1) concluded that the salt content of leaf tissues is specific in the varieties of cotton grown in Egypt.

It seems probable that differences between the individual varieties of the Egyptian type and between the individual varieties of the upland type may be demonstrated, but this will require more extensive and more refined experimentation for final proof. Constants for one series of sea-island cotton are given, but since other investigations on this type are under way the results will not be discussed in detail here.

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THE USE OF VACUUM FOR INSECT CONTROL ¹

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INTRODUCTION

The use of high vacuum for the control of insects seems never to have been considered seriously by the commercial world. The recent development and exploitation by private interests of a vacuum chamber intended for the treatment of certain warehoused commodities as a part of the equipment of a modern storage warehouse has aroused much interest in business circles and has given the writers an opportunity to make tests reported upon in this paper.

HISTORICAL

The possibility of killing insects by depriving them of air was conceived at least 250 years ago when the eminent scientist Robert Boyle (2)² invented a mechanical air pump³ capable of exhausting a large proportion of the air from small receptacles. Boyle studied the effects of vacuums upon various forms of life. Unfortunately, from his published accounts one can not know the exact degree of vacuum employed. Although the vacuum obtained was by no means perfect, it proved effective in killing many forms with which he experimented. Boyle's experiments are of great interest, but lack of information makes it impossible to interpret them satisfactorily.

Jousset de Bellesme (5, p. 710), experimenting with vacuum in 1880, concluded that Hymenoptera could survive in vacuum for 5 or 6 hours at a temperature of 20° C. and Blattidae (cockroaches) for 2 hours at a temperature of 12° C.

Cole (3, p. 69-71), in 1906, in an account of the bionomics of the grain weevils, records experiments with vacuum upon *Sitophilus oryza*. According to his statement, adults of the rice weevil fed and oviposited when confined in a partial vacuum, and 5 out of the 10 weevils experimented with were alive at the end of 23 days. In the experiment the mercury of the gauge was reduced to 5 inches. In a second experiment, with the mercury reduced to 1 inch, 5 out of 10 adults were alive after an exposure to the vacuum for 15 days.

Nagel (7), in 1921, published an account of experiments conducted for the control of *Anobium striatum* Oliv. and reported that larvae of this pest were unharmed by exposure to vacuum for 24 hours.

The use of the vacuum in connection with fumigation work is well known and will not be discussed in this paper. Its use in connection with heat was tried by Mackie (6) for the control of the tobacco beetle infesting cigars. Mackie says (6, p. 135):

The process in question consists of heating the tobacco to a certain degree after which the air is pumped out until a 28-inch vacuum is registered by the vacuumeter. The material being heated higher than the vapor tension point

¹ Received for publication June 16, 1925; issued January, 1926.

² Reference is made by number (italic) to "Literature cited," p. 1041.

³ The first mechanical air pump was invented by Otto von Guericke in 1654, an account of his experiments with vacuums having been published in 1672 (4). He experimented with birds and fish in vacuum but apparently did no work with insects. Boyle improved upon Von Guericke's pump and published an account of his experiments in 1670.

of water under this pressure, this causes the water content of all bodies comprehended therein to change to a gaseous form, and thus all insects are killed.

Brief notes on the effect on insects of a vacuum of 24 to 28 inches were given in a paper by the writers (1) at the Annual Convention of the American Warehousemen's Association, held in Chicago in December, 1924.

APPARATUS USED

The apparatus used by the writers in their experiments consisted of a concrete vault, 8 by 8 by 8 feet, and a large glass bell jar having a cubic content of approximately 825 cubic inches. The air was pumped from these two containers by means of a dry vacuum pump. In the case of the large concrete vault a vacuum of 28 inches was obtained within a period of approximately one and a half hours. An automatic control then cut off the motor, the pump remaining idle until the vacuum fell to 24 inches, when the control device automatically started the motor again. The small amount of leakage that occurred allowed the vacuum to fall from 28 inches to 24 inches in from 4 to 6 hours. In the case of the bell jar a 29-inch vacuum was obtained within 30 seconds of the time the pump was started, but little leakage occurred and no control device was used; whenever necessary, the pump was started by hand.

BELL-JAR EXPERIMENTS

EXPERIMENT NO. 1

In the first experiment specimens of 19 different species of insects affecting stored products were used. In some cases two or more stages of the insects were used, in others only the adults; in all cases from 10 to 50 specimens were used in each lot treated. The insects were subjected to a vacuum varying from 26 inches to 29 inches for periods of 1 day, 2 days, and 4 days, respectively. The temperature during the experimental period varied between 60° and 70° F. and the barometer reading for the period varied between 29.3 and 29.7 inches.

TABLE I.—Effect of vacuum varying from 26 to 29 inches upon insects in bell jar of 825 cubic inches capacity

Insect	Killed in 1 day				Killed in 2 days				Killed in 4 days			
	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
Alphitobius piceus Oliv.	-----		100	100	-----	100	-----	100	-----	100	-----	100
Anthrenus fasciatus Hbst.	-----	50	100	-----	-----	100	100	-----	-----	100	100	100
Attagenus piceus Oliv.	-----	50	-----	100	-----	100	100	100	-----	100	100	100
Cryptolestes pusillus Schon.	-----	-----	-----	100	-----	100	-----	100	-----	100	100	100
Dermestes vulpinus Fab.	-----	100	-----	100	-----	100	-----	100	-----	100	100	100
Ephestia kuehniella Zell.	-----	100	100	100	-----	100	100	100	-----	100	100	100
Necrobia rufipes De Geer.	-----	-----	-----	100	-----	-----	-----	100	-----	-----	-----	100
Oryzaephilus surinamensis L.	-----	-----	-----	100	-----	-----	-----	100	-----	100	-----	100
Plodia interpunctella Hbn.	-----	100	100	100	-----	100	-----	100	-----	100	100	100
Silvanus gemellatus Duv.	-----	-----	-----	100	-----	-----	-----	100	-----	100	100	100
Sitophilus oryza L.	-----	-----	-----	100	-----	-----	-----	100	-----	-----	-----	100
Sitophilus granarius L.	-----	-----	-----	100	-----	-----	-----	100	-----	-----	-----	100
Tenebrio obscurus Fab.	-----	-----	-----	100	-----	-----	-----	100	-----	100	-----	100
Tenebroides mauritanicus L.	-----	80	-----	50	-----	100	-----	100	-----	100	-----	100
Tineola biselliella Hum.	10	50	100	100	40	100	100	100	100	100	100	100
Tinea pellionella L.	-----	40	-----	-----	-----	100	-----	-----	-----	100	-----	-----
Tribolium confusum Duv.	-----	-----	-----	100	-----	-----	-----	100	-----	100	100	100
Tribolium ferrugineum Fab.	-----	-----	-----	100	-----	-----	-----	100	-----	100	100	100
Trogoderma tarsale Melsh.	-----	0	-----	-----	-----	20	-----	-----	-----	100	100	100

As indicated by the data of Table I, an exposure of 24 hours was fatal to nearly all the insects treated. Larvae of the dermestid beetles proved quite resistant, only 50 per cent of those of *Anthrenus fasciatus* and *Attagenus piceus*, and none of *Trogoderma tarsale* being killed. The eggs of the webbing clothes moth, *Tineola biselliella*, and the larvae of both this clothes moth and the case-making clothes moth, *Tinea pellionella*, showed much resistance.

A two-day exposure killed all specimens treated with the exception of the eggs of *Tineola biselliella*, of which only 40 per cent were killed, and the larvae of *Trogoderma tarsale*, of which only 20 per cent were killed. A four-day exposure killed all stages of all insects.

EXPERIMENT NO. 2

In the second experiment the same species of insect pests were used, except that *Gnathocerus cornutus* and *G. maxillosus* displaced *Sitophilus granarius*. A 29-inch vacuum was obtained and the vacuum was not allowed to fall below 28 inches at any time during the course of the experiment. The barometer reading was 29.3 inches and the temperature for the period varied between 60° and 70° F.

As shown in Table II, a large percentage of the insects treated were killed by a seven-hour exposure. Adults of all species were killed, but the larvae of *Tenebrio*, *Tineola*, *Tinea*, and *Trogoderma* were apparently but little affected by the seven-hour exposure, whereas 70 per cent of larvae of *Ephestia* and 80 per cent of larvae of *Plodia* were killed. A one-day exposure to this vacuum killed all stages of all insects experimented with, with the exception of the larvae of *Trogoderma tarsale*, 50 per cent only of which were killed, and the eggs of *Tineola biselliella*, 90 per cent of which survived.

TABLE II.—Effect of vacuum varying from 28 to 29 inches upon insects in bell jar of 825 cubic inches capacity

Insect	Killed in seven hours			Killed in one day			
	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
<i>Alphitobius piceus</i> Oliv.	-----	-----	100	-----	-----	-----	100
<i>Anthrenus fasciatus</i> Hbst.	-----	-----	100	-----	100	100	100
<i>Attagenus piceus</i> Oliv.	100	100	100	-----	100	100	100
<i>Cryptolestes pusillus</i> Schon.	-----	-----	100	-----	100	100	100
<i>Dermestes vulpinus</i> Fab.	100	-----	100	-----	100	-----	100
<i>Ephestia kuehniella</i> Zell.	70	-----	100	-----	100	100	100
<i>Gnathocerus cornutus</i> Fab.	-----	-----	100	-----	-----	-----	100
<i>Gnathocerus maxillosus</i> Fab.	-----	-----	100	-----	-----	-----	100
<i>Necrobia rufipes</i> De Geer.	-----	-----	100	-----	-----	-----	100
<i>Oryzaephilus surinamensis</i> L.	-----	-----	100	-----	-----	-----	100
<i>Plodia interpunctella</i> Hbn.	80	-----	100	-----	100	100	100
<i>Silvanus gemellatus</i> Duv.	-----	-----	100	-----	-----	-----	100
<i>Sitophilus oryza</i> L.	-----	-----	100	-----	-----	-----	100
<i>Tenebrio obscurus</i> Fab.	0	-----	100	-----	-----	-----	100
<i>Tenebroides mauritanicus</i> L.	-----	-----	-----	-----	100	-----	100
<i>Tineola biselliella</i> Hum.	0	-----	-----	10	100	100	100
<i>Tinea pellionella</i> L.	0	-----	-----	-----	100	-----	-----
<i>Tribolium confusum</i> Duv.	-----	-----	100	-----	-----	-----	100
<i>Tribolium ferrugineum</i> Fab.	-----	-----	100	-----	-----	-----	100
<i>Trogoderma tarsale</i> Melsh.	0	-----	-----	-----	50	-----	-----

VAULT EXPERIMENTS

Specimens of 20 species of insects were used in the experiments conducted in the concrete vault. They were confined in pill boxes or glass vials stoppered with cotton, and individuals of each species were placed on a ledge of an observation window so that their actions could be observed at all times. A vacuum of from 24 inches to 28 inches was maintained automatically throughout the experiment. The barometer reading for the period varied between 29.3 and 29.7 inches; the temperature varied between 60° and 70° F. At the end of three days specimens of all species of insects were removed and examined.⁴ Other specimens of all species were removed at the end of 4 days, 5 days, 6 days, and 7 days, respectively.

The data in Table III indicate that exposure to a vacuum varying from 24 to 28 inches for three days killed a large proportion of the insects. A very considerable number of the coleopterous larvae survived, only 20 per cent of those of *Trogoderma tarsale* being killed, and 60 per cent of those of *Tenebrio obscurus*. The softer-bodied larvae of *Dermestes vulpinus* and the lepidopterous larvae were killed. The adults which escaped belong to the small grain-infesting species, with the exception of adults of *Alphitobius* which are larger by comparison.

TABLE III.—Effect of vacuum varying from 24 to 28 inches upon insects in a concrete vault containing 512 cubic feet

Insect	Killed in three days				Killed in four days				Killed in five days				Killed in six days				Killed in seven days			
	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
Alphitobius piceus Oliv	---	90	---	86	---	90	---	86	---	100	---	96	---	100	---	98	---	100	---	100
Anthrenus fasciatus Hbst	---	92	100	100	---	100	100	100	---	100	100	100	---	100	100	100	---	100	100	100
Attagenus piceus Oliv	---	66	100	100	---	78	100	100	---	78	100	100	---	80	100	100	---	95	100	100
Cryptolestes pusillus Schon	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Dermestes vulpinus Fab	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100
Ephestia kuehniella Zell	---	100	100	100	---	100	100	100	---	100	100	100	---	100	---	100	---	100	---	100
Gnathocerus cornutus Fab	---	90	---	98	---	100	---	100	---	100	---	100	---	100	100	100	---	100	100	100
Gnathocerus maxillosus Fab	---	90	---	94	---	100	---	100	---	100	---	100	---	---	---	100	---	---	---	100
Necrobia rufipes De Geer	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Oryzaephilus surinamensis L	---	---	---	50	---	---	---	65	---	---	---	75	---	---	---	90	---	---	---	100
Plodia interpunctella Hbn	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Silvanus gemellatus Duv	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Sitophilus oryza L	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Sitophilus granarius L	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Tenebrio obscurus Fab	---	60	---	100	---	60	---	100	---	80	---	100	---	80	---	100	---	80	---	100
Tineola biselliella Hum	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Tinea pellionella L	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---
Tribolium confusum Duv	---	---	---	98	---	---	---	99	---	---	---	100	---	---	---	100	---	---	---	100
Tribolium ferrugineum Fab	---	---	---	99	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Trogoderma tarsale Melsh	---	20	---	---	---	20	---	---	---	20	---	---	---	30	---	---	---	30	---	---

The data indicate that exposure for 4, 5, 6, and 7 days did not kill all individuals. It is interesting to note that adults of *Alphitobius piceus* and *Oryzaephilus surinamensis* were not all killed by an exposure of 6 days (although the last ones succumbed on the seventh

⁴ All treated specimens were held for two weeks after the experiment and were examined at frequent intervals to see if any would recover.

day), and that 7 days' exposure did not kill all larvae of *Attagenus piceus*, *Tenebrio obscurus*, and *Trogoderma tarsale*.

Additional experiments, in which specimens of these insects were exposed to the same vacuum for two days only, gave the following results: Larvae of *Plodia interpunctella*, *Ephestia kuehniella*, and *Pyralis farinalis*, and adults of *Sitophilus oryza* and *S. granarius* were all killed. Many larvae and adults of the clothes moth *Tineola biselliella* were killed, but specimens of the other insects were apparently little affected.

GENERAL OBSERVATIONS

By using the glass bell jar and the observation windows of the vault the activity of the insects in the vacuum chamber could be readily observed. It was noted that when the insects were subjected to a 29-inch vacuum, all movement ceased in all stages of all species within two minutes after the pump was started. Beetles and moths fell upon their backs with every appearance of being dead. If the air was restored within a short time the insects regained their activity and appeared little the worse for their experience, except in the case of adults of the meal worm *Tenebrio obscurus*. The meal-worm beetles when treated to this vacuum for only 10 minutes showed feeble movements after being restored to the air, but died within a few hours thereafter.

When insects were exposed to the 24 to 28 inch vacuum many became inactive soon after the vacuum was obtained but others remained slightly active for several days.

In general, the adult and pupal stages of the insects experimented with were more susceptible to the effect of vacuum than were their larval stages. Lepidopterous insects were for the most part rather easily killed. Treated specimens of the larvae of these and other insects were stiff and brittle and appeared to have been thoroughly dried out by the process. To test the effect of vacuum on water, a small amount was placed in a glass vial under the bell jar and subjected to a 29-inch vacuum; the water boiled violently until the air had been removed from it.

Candy, which is often infested with insects, was also treated and the effect noted. Chocolate creams were broken open by the force of the vacuum, although chocolate-covered nuts were apparently uninjured.

Insects sealed in cartons of breakfast foods and buried in rolls of clothing or in upholstered furniture were killed as quickly as though they had been exposed in pill boxes or cotton-stoppered glass vials.

SUMMARY

A vacuum of 28 to 29 inches, maintained for 7 hours in a bell jar of 825 cubic inches capacity when the temperature ranged from 60° to 70° F. and the barometer read 29.3 inches, killed all adults of *Alphitobius piceus*, *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Gnathocerus cornutus*, *G. maxillosus*, *Necrobia rufipes*, *Oryzaephilus surinamensis*, *Plodia interpunctella*, *Silvanus gemellatus*, *Sitophilus oryza*, *Tenebrio obscurus*, *Tribolium confusum*, and *T. ferrugineum*. It killed all pupae of

Attagenus piceus, and all larvae of *A. piceus* and *Dermestes vulpinus*, 70 per cent of the larvae of *Ephestia kuehniella* and 80 per cent of the larvae of *Plodia interpunctella*. Larvae of *Tenebrio obscurus*, *Tineola biselliella*, *Tinea pellionella*, and *Trogoderma tarsale* were apparently not affected. Exposure for 24 hours to this vacuum killed all adults, pupae, and larvae except 50 per cent of the larvae of *Trogoderma tarsale*. Eggs of none of the species were to be had except of *Tineola biselliella*, and of these only 10 per cent were killed.

A vacuum of 26 to 29 inches in the glass bell jar, with the temperature ranging from 60° to 70° F., and the barometer from 29.3 to 29.7 inches, maintained for four days, killed all adults of *Alphitobius piceus*, *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Necrobia rufipes*, *Oryzaephilus surinamensis*, *Plodia interpunctella*, *Silvanus gemellatus*, *Sitophilus oryza*, *S. granarius*, *Tenebrio obscurus*, *Tenebroides mauritanicus*, *Tineola biselliella*, *Tribolium confusum*, *T. ferrugineum*, and *Trogoderma tarsale*. It killed all pupae used in the experiment, including those of *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Plodia interpunctella*, *Silvanus gemellatus*, *Tineola biselliella*, *Tribolium confusum*, *T. ferrugineum*, and *Trogoderma tarsale*. All larvae of the 18 species above mentioned except *Necrobia* and *Sitophilus* (which were not included in the experiment) and the larvae of *Tinea pellionella* were killed, and all the eggs of *Tineola biselliella*. Eggs of this species were the only eggs available for experimental purposes.

Exposure of two days to the 26 to 29 inch vacuum killed all forms included in the experiments except 80 per cent of the larvae of *Trogoderma tarsale* and 60 per cent of the eggs of *Tineola biselliella*. Exposure for only one day killed a large proportion of the forms included except 50 per cent of the adults of *Tenebroides mauritanicus*, 50 per cent of the larvae of *Anthrenus fasciatus*, *Attagenus piceus*, and *Tineola biselliella*, 40 per cent of the larvae of *Tinea pellionella*, 80 per cent of the larvae of *Tenebroides mauritanicus*, none of *Trogoderma tarsale*, and only 10 per cent of the eggs of *Tineola biselliella*.

A vacuum of 24 to 28 inches, maintained in a concrete vault 8 by 8 by 8 feet, when the temperature varied between 60° and 70° F., and the barometer readings were between 29.3 and 29.7 inches, gave results which indicate that the usual fabric pests troublesome in storage warehouses can be killed by the vacuum treatment. Eggs, larvae, pupae, and adults of the common or webbing clothes moth, *Tineola biselliella*, were dead at the end of exposures of 3, 4, 5, 6, and 7 days. All pupae and adults of the carpet beetles *Attagenus piceus* and *Anthrenus fasciatus* were dead at the end of the same exposures. Ninety-two per cent of the larvae of *Anthrenus fasciatus* were killed by the end of an exposure of 3 days, and all after 4, 5, 6, and 7 days. The larvae of *Attagenus piceus* were most resistant; only 66 per cent being killed by an exposure of 3 days, 78 per cent by exposures of 4 and 5 days, 80 per cent by an exposure of 6 days, and 95 per cent by an exposure of 7 days. *Oryzaephilus surinamensis* was the most resistant of all species tested in the adult stage, 50, 65, 75, 90, and 100 per cent being killed by exposures of 3, 4, 5, 6, and 7 days, respectively. The larvae of *Attagenus piceus*, *Tenebrio*

obscurus, and *Trogoderma tarsale* were the most resistant of the larval forms used, 3 days' exposure killing only 66, 60, and 20 per cent, respectively; and 7 days' exposure killing 95, 80, and 30 per cent, respectively.

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THE RELATION OF SIZE OF KERNELS IN SWEET CORN TO EVENNESS OF MATURITY¹

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INTRODUCTION

Commercial canners have found a wide range in the degree of maturity of sweet-corn ears in the canning season. When the majority of the ears are ready to harvest some have already become too hard and tough, and others are still too young and watery to give the pack the proper consistency. This unevenness of maturity varies with the variety and appears to be worst in the small or narrow grain types. It is particularly troublesome in the Country Gentleman, where the slender "shoe-peg" kernel predominates. It causes a lack of uniformity in the canning condition of the corn, which results in a pack of poor quality unless the ears are properly graded, an operation which adds to the expense of handling the crop since the grading must be done by hand. In order that these expensive variations may be avoided, it is important that as much of the crop in each field mature at the same time as possible.

In 1918 the Indiana Canners' Association requested the horticultural department of the Purdue station to study the sweet-corn problem with a view to developing better strains of the standard canning varieties and ones that would be more suitable to Indiana conditions. The work reported here is a part of this larger problem.

For several years, while sweet-corn seed was being tested for germination and freedom from disease, it was observed that the small kernels produced slenderer sprouts with smaller root systems than the larger kernels. This was true whether the kernels were picked from the same ears or from bulk-shelled samples. The question then arose as to whether or not this condition affected the rate of growth and time of maturity of the progeny. In the fall of 1920 large and small kernels of the same varieties were planted in the greenhouse to determine whether the differences noted in the germinator would be found in soil-grown plants. Very striking differences both in size of seedlings and in rate of growth developed, and the investigation was enlarged and continued for more than two years. During this time a large number of observations and experiments were made under greenhouse and field conditions, and these will be discussed in the following pages.

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REVIEW OF LITERATURE

Investigators working with a great many varieties have found that for the most part large, heavy seeds produce plants of greater size and higher yield than small ones, and the crop is more uniform. Other investigators, however, have found the results of their work conflicting or inconclusive. A brief résumé of published data is given here in order that the scope of the investigations may be understood, but it is not intended to include all that have been reported.

Dehérain and Dupont (3)³ claim that a definite advantage in favor of heavier seed shows only when the difference in weight is great. Myers (10), working with wheat, found his results inconclusive. Leighty (8) criticizes the method of selecting large seed without knowing something of the characteristics of the mother plant. Love (9), in his work with wheat and oats, finds that the heaviest grains come from the tallest and heaviest yielding plants. Johannsen (5), from his studies of the inheritance of weight of bean seeds, concludes that the heaviest daughter beans are the progeny of the heaviest mother beans, but that this does not necessarily hold in a pure line. DeVries (15), on the other hand, thinks that size and weight of seeds are the result of nutrition in the broad sense rather than of inheritance.

Studies have also been made of seeds in relation to their specific gravity. Haberlandt's (4) work with wheat and oats shows that the denser kernels yield heavier returns in grain and the lighter kernels yield greater quantities of straw. Wollny (16) maintains that its absolute weight and not its specific gravity is the true index to the value of grain. Clark (1) finds that size of seed is the most important factor for selecting within a variety, that is, the heaviest seed in a given size (volume) gives the best results.

In studying the combined effects of size and specific gravity, Sanborn (14) found that the yield from the lighter grains of wheat is greater than from the heavier ones. Degrully (2), working with corn, discarded all of the very small and poorly formed kernels and separated out the lightest one-fourth of the remainder by means of sodium nitrate solution. The difference in results in favor of the heavy kernels was very striking. He also found that wheat seedlings from heavy grains are greener, more vigorous, and greatly superior to those from lighter grains. These differences, he believes, are the result of some factor inherent within the seed. Kiesselbach and Helm (6) found that the total "sprout values" of unselected grains and large and small grains of wheat were, respectively, 100, 123, and 100.88. These figures indicate a rather close relationship between the size of seed and its sprout value. Kisselbach and Cook (7) working with corn find that increases in kernel weight may be due to two causes: (1) heterosis (11.8 per cent) as in crosses in selfed strains, and (2) a change in type of endosperm (19.9 per cent), as sweet corn crossed with dent or flint varieties. Commercial dent varieties, being already heterozygous, respond relatively little (0.2 per cent increase) to the immediate effect of foreign pollen as a result of heterosis. Pearl and Surface (11), working with corn, found that there is a marked tendency for the plants which were relatively small

³ Reference is made by number (italic) to "Literature cited," page 1052.

at the beginning of the season to remain, on an average, relatively small throughout most of the season; extreme types at the beginning of the season tend strongly to remain extreme types during the whole season. This behavior is thought to be the result of internal rather than external causes. Reed (12) shows that the seedlings of *Helianthus* follow the same tendency as that described by Pearl and Surface for corn. Plants which were small at the beginning were generally small at maturity; those which were large at the beginning usually remained so throughout the season. He also concluded that this behavior is the result of inherent tendencies rather than of external causes. In studying garden beans Renich (13) found that large, heavy seeds give the best growth and the largest plants. The range of variation in size and weight of seedlings is wider between those from small and medium-sized seeds than between the medium-sized and large seeds.

MATERIALS AND METHODS

Since Country Gentleman is one of the varieties of sweet corn that varies most widely in the size of its kernels, it was chosen for most of the experiments, although other varieties were used. An examination of the kernels showed that on the same ear they varied in size from the very slender "shoe-peg" type to the larger and broader crowned types (pl. 1, A). These large and small types of kernels gave, respectively, large and small seedlings in the germinator with correspondingly large and small root systems. Disease-free seed was used, and it was certain that these differences were the result of natural causes and in no way attributable to disease.

GREENHOUSE TESTS

In order that their behavior in the soil might be observed, the kernels of each type were planted in the greenhouse early in the spring of 1921 before the weather was warm enough for field planting (pl. 1, B). No difference was noted in the time required for the two types to germinate and the seedlings to appear. During the first few days the seedlings grew rapidly, but even at this time marked differences were evident between the two sets of progeny; those from the small kernels were slender with narrow leaves, while those from the large kernels were stocky and vigorous and their leaves were wide.

The greenhouse tests were repeated in the spring of 1922, a larger number of kernels of each grade being used than had previously been employed. These experiments were made with the double purpose of determining the rate of germination of the two grades and the relative size of the progeny. The kernels of both types germinated at about the same time, and both varied somewhat, but neither had a distinct advantage over the other. Representative stalks of both types were transplanted to the field as soon as weather conditions would permit the making of careful growth studies. The data for these stalks are presented later in the paper.

FIELD TESTS

In 1921 a large number of kernels of both types were picked out by hand and kept separate for use in field tests. A part of the samples were taken from individual ears and the rest from a shelled bulk

sample. Where the samples were picked from the ears the kernels were taken from all parts, so that they would represent the whole ear rather than separate portions of it. Those samples taken from the same ears were planted in parallel rows, adjacent to each other (pl. 1, C). A test of 13 ears was included in this experiment, the results of which are shown in Table I.

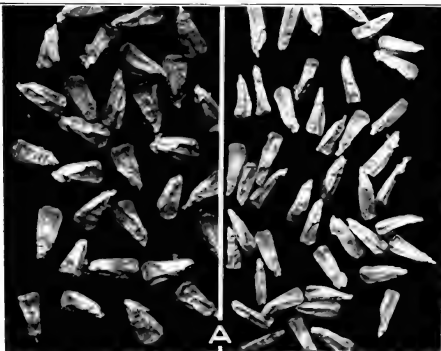
TABLE I.—Field performance of large and small kernels taken from the same ears of sweet corn

Row No.	Ear No.	Kernel grade	Number of kernels planted	Percentage stand	Relative size of stalks	Days to full tassel	Stage of sweet corn
1	1	{Large	28	85.7	Large	47	Canning.
2		{Small	27	40.7	Small	54	Premilk.
3	2	{Large	30	76.6	Large	51	Canning.
4		{Small	29	37.9	Small	54	Premilk.
5	3	{Large	31	96.8	Large	51	Canning.
6		{Small	27	70.4	Small	56	Premilk.
7	4	{Large	28	92.8	Large	51	Canning.
8		{Small	26	76.9	Small	54	Premilk+ ^a .
9	5	{Large	27	96.7	Large	51	Canning+.
10		{Small	27	77.7	Small	56	Premilk+.
11	6	{Large	29	93.1	Large	51	Canning.
12		{Small	28	85.7	Medium	54	Premilk+.
13	7	{Large	27	92.6	Large	47	Canning+.
14		{Small	30	86.6	Medium	50	Canning.
15	8	{Large	30	93.1	Large	54	Canning.
16		{Small	27	90.0	Small	57	Premilk+.
17	9	{Large	30	96.6	Large	47	Dough—.
18		{Small	27	85.2	Small	55	Canning—.
19	10	{Large	25	80.0	Large	51	Canning.
20		{Small	29	96.5	Small	54	Canning—.
21	11	{Large	25	84.0	Large	47	Canning.
22		{Small	27	85.2	Small	51	Premilk.
23	12	{Large	28	92.8	Large	47	Dough—.
24		{Small	24	91.7	Medium	47	Canning.
25	13	{Large	22	95.4	Large	47	Canning.
26		{Small	22	72.2	Small	53	Premilk.

^a The plus and minus signs are used to indicate conditions of maturity "greater than" or "less than," respectively, the state of maturity with which they are used. Thus "premlk +" means that the corn is slightly past the premlk stage, but not yet in the best canning condition, as determined by the thumb-nail test.

^b The kernels in the "small" grade were distinctly smaller than those in the "large" grade, but neither as slender nor as light as those from the other ears; in a bulk sample they would be classed as medium-sized. Consequently, the stalks from these kernels were less delayed in beginning their growth, and there was less difference in time of reaching the canning stage.

The most significant data in Table I are those for percentage of stand, days of full tassel, relative size of stalks, and condition of the corn when the ears on the large stalks were ready to pick. In studying the stand or germination of the seeds for the various ears and grades it was found that the large kernels germinated better than the small ones. The large seed produced larger and stockier plants than the small ones, and the greater the difference in the size of the kernels the greater the difference in the size of the plant. These differences were plainly evident from the beginning and remained so until the stalks were mature. The large stalks reached full tassel about five days before the small ones and the ears reached the canning stage with about the same time interval. When the ears of the large stalks were in the best canning condition, those on the small stalks were so tender and watery that the corn was unfit for use, and it would have taken about five days longer under the existing conditions for it to reach the same stage.



A.—Large and small kernels of Country Gentleman sweet corn. These sizes are found in all parts of the same ear and are very common in this variety.

B.—Stalks produced by the kernels shown in A. The large stalks on the right were produced by the large kernels. The small ones in the middle were produced by the small kernels, and those on the left by a mixture of both types. Note the small stalks in the foreground and the large ones in the rear of this row.

C.—Parallel rows planted with large and small kernels from the same ears. Rows 1 and 2 (left to right) are from ear No. 1, and rows 3 and 4 are from ear No. 2, the data for which are given in Table I. Note the correlation between size of kernels and size of stalks.

Another field test was made at Shelbyville, Ind., in 1922, in cooperation with a commercial canning company. This test was conducted on a larger scale and bulk seed was used instead of that selected from individual ears. The corn used was a strain of Country Gentleman which had been grown and selected under local conditions for four years. It had become fairly well acclimated in this time and was of good type for the variety. A sufficient number of large and small kernels were hand picked to plant a half acre each, and in addition to these plots one of equal size was planted with ungraded seed from the same source to serve as a basis for comparison.

The soil was a very rich, uniform, black loam. It was well drained and in a fine state of cultivation. The corn was drilled with a two-horse corn planter, the smallest plates being used.

When the corn came up the stand was very good. The stalks in the plot planted with large kernels were uniform in vigor, size, and color. There were practically no small or slender stalks in the whole plot; they were vigorous and stocky, and dark green in color.

The stalks in the plot planted with small seed were much shorter and slenderer than those from the large kernels. They were fairly uniform in color, but varied somewhat in size. (Note the similarity of the following observations to the data given in Table III.)

A wide range of variation was evident in the plot planted with the ungraded bulk seed. The stalks ranged in size from the small, slender types to the large, stocky ones, with various intermediate types between.

At tasseling time the stalks from the large kernels came into full tassel evenly and about five days in advance of those from the small ones; in fact, it was possible to distinguish these rows a long distance from the field by this earlier and more uniform development. The stalks from the small kernels were distinctly smaller and later at this time; they were tasseling irregularly and only about 20 per cent had reached the tassel stage.

The plot planted with the ungraded seed was also very irregular. It was composed of a mixture of stalks varying in size and maturity from those in full tassel to those in which the tassels were just beginning to form.

HARVEST DATA

Outstanding differences were noted in the plots at harvest time. These consisted chiefly in time and evenness of maturity and size of stalks and yields. The plants from the large seeds were larger and more uniform in maturity than any in the series, about 95 per cent having reached maturity at the same time. They were well developed and the kernels were of fair size and deep. A good many of the stalks bore two full-sized ears.

The stalks from the small kernels were shorter and slenderer than those from the large ones and were uneven in maturity. A few were as mature as those in the plot from the large seed, some had quite recently come into full silk, but the majority were just passing out of the premilk stage, that is to say, they were about five days later than the plants from the large seed.

The plot planted with ungraded seed was also very irregular in the size of its stalks and the maturity of its ears. The latter ranged

from the canning stage through all other stages down to the fresh silk. Two pickings were made in each plot. Nearly all of the corn was removed the first time from the plot planted with large kernels, but about half of the yield of the other two plots remained for the second picking.

TABLE II.—*Field performance of large and small kernels from the same bulk sample*

Plot	Number of bearing stalks	Percent- age of barren- ness	Yield	Average yield per plant
			<i>Tons per acre</i>	
Ungraded kernels.....	2,775	16	2.40	1.70
Large kernels.....	2,406	8	2.53	2.14
Small kernels.....	3,019	25	2.63	1.60

Table II gives a summary of the field performance of the three plots. The total stand varied with the size of the seed because the planter was unable to drop the small kernels as evenly as the larger ones. It will be noted that the percentage of barren and unproductive stalks was smallest among the large kernels, largest among the small ones, and intermediate in the plot of ungraded seed. The yield per acre was greatest in the plot planted with small kernels owing to the greater number of plants. The average yield per plant also varies with the size of the seed. The large kernels yielded 0.54 pound per plant more than the small kernels and 0.44 pound more than the ungraded seed. The average yield for the ungraded seed was higher than that for the small seed because of the large kernels contained in it. Many of these kernels produced two ears, just as did those in the plot planted with large seed, a fact which tended to raise the average yield per stalk.

PLANT-GROWTH MEASUREMENTS

In order to obtain detailed information on the relative rate of growth of cornstalks from large and small kernels, the following data were carefully collected. Thirty representative stalks from both types of kernels were selected from a large number which were started in the greenhouse. These were transplanted to the field and records of growth were taken at weekly intervals throughout their development. The data consisted in height of stalk, diameter of base, time of tasseling, time of shedding pollen, time of appearance of silk, time of arrival at full silk, time at which the ear reached the canning stage, yield of plant, size of ears, size of kernels, and number of tillers (suckers) for each type of plant. As many of these data as possible are presented in Figure 1, some are given in Table III, and the remainder are discussed in the following paragraphs.

Figure 1 shows the rate of growth, time of tasseling, final height, period of pollen shedding, time at which the ears came into full silk, and time at which they reached the canning stage. It so happened that transplanting was done at the beginning of a drouthy period, and as long as the drought continued the plants made little growth.

The large stalks suffered less than the small ones and the line of growth shows a greater angle of elevation; this period is represented by the flattened portion of the graph. The lines of growth separate immediately after the corn comes up and they never again unite. Large seeds have more reserve food material than small ones, and this seems to be the factor which determines the difference in the initial growth. The germinator showed, as previously stated, that the root systems of the plants from the large kernels were much larger than from the small ones and these provide a means of supplying the plants with greater quantities of food materials from the soil. It is believed that these two factors are responsible for the difference in growth and time of development in the two types.

Figure 1 shows also that there is a difference of five days in tasseling, silking, and reaching the canning stage between corn grown from large and corn grown from small kernels, a fact which accounts for

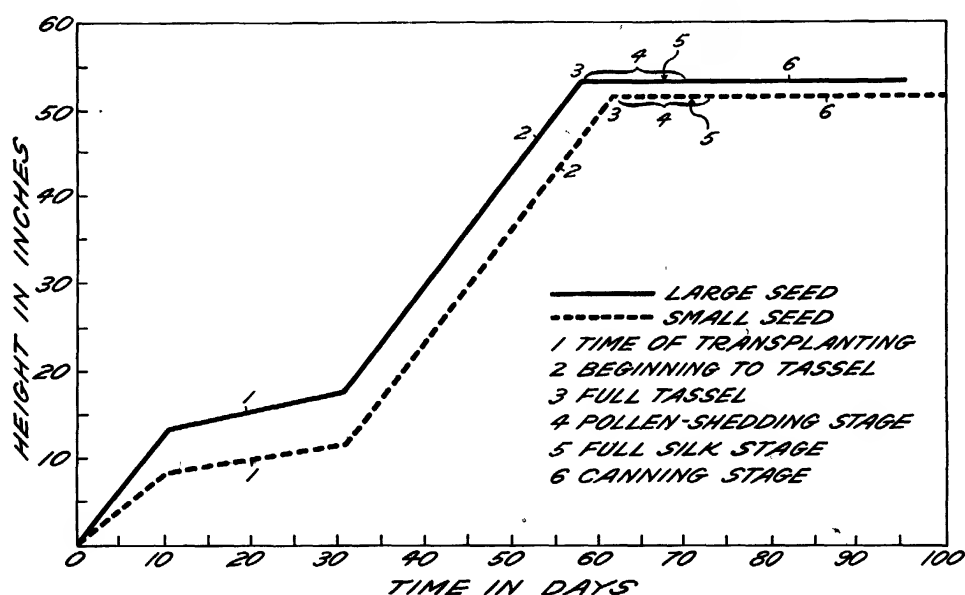


FIG. 1.—The relation of kernel size to evenness of maturity

the unevenness of maturity found in fields of sweet corn grown for canning purposes.

The height of the plant was measured by taking the highest point reached when the leaves were held upright, except the final measurement, which was taken from the tip of the tassel when it had stopped growing. The diameter of the stalks at the base was taken in the middle of the first internode and shows a constant enlargement in proportion to the height of the plant.

Table III gives a part of the data collected in the plant growth studies which could not be expressed in the preceding graph. The results reported in the table are very consistent and substantiate the observations made at Shelbyville, Ind., the same year, and also the results obtained at the station the previous year. At 9 days of age the plants from the large seed averaged 4.15 inches taller and 0.048 inch greater in diameter than the average from the small seed and 2.05 inches taller and 0.023 inch greater in diameter than the average from the ungraded seed.

At maturity, after all growth had stopped, there was still some difference in size between the stalks of the different grades, although not so much as there had been earlier in the season. The stalks from the large seed averaged 1.6 inches taller and 0.05 inch greater in diameter than those from the small seed and 0.6 inch taller and 0.02 inch greater in diameter than those from the ungraded seed.

By reference to Figure 1 it is apparent that stalks from the small seed showed no tendency to catch up with those from the large seed until after the large ones had almost matured their ears; consequently the ears from the small grade were later in maturing. This fact is emphasized by the data presented in Table III, which shows that when the corn from the large grade of seed was in full tassel only 20 per cent from the small grade and 50 per cent from the ungraded seed had reached the tassel stage. Also, when the corn from the large seed was in full silk that from the small grade was only 10 per cent in silk and that from the ungraded seed but 25 per cent. Finally, when the ears on the stalks from large seed were harvested, 95 per cent were in the best canning stage, whereas but 15 per cent from the small seed and 30 per cent from the ungraded seed had reached that stage. These data indicate that the variation in maturity of corn is due to the difference in size of the parent seed.

TABLE III.—*Summary of plant-growth data in sweet corn*

	Grade of seed		
	Large	Small	Ungraded
Average size of stalks 9 days of age:	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>
Height.....	13.2	9.05	11.15
Diameter.....	.173	.125	.150
Average size of same stalks at maturity:			
Height.....	53.4	51.8	52.7
Diameter.....	.75	.70	.73
Percentage of stalks in full tassel when large grade was in full tassel.....	100	20	50
Percentage of stalks in full silk when large grade was in full silk.....	100	10	25
Percentage of corn mature when large grade was gathered.....	95	15	30

In yield per plant the stalks from large seed gave slightly more than those from small ones, which was occasioned by the fact that two yielded more than one ear. The size of the ears in each type was about the same. In the field test at Shelbyville the stalks from the large kernels yielded 1.3 times as much as those from the small ones.

There was no difference in the number of tillers between the two types. The average number for the large seed was 2.13 and for the small seed, 2.15.

The ears were examined to determine whether the size of the parent kernel influences the size of the kernel in the offspring, and it was found that the sizes varied in both types throughout the same range, the average being about the same. It was therefore concluded that the progeny of large kernels is just as desirable for packing as that from small ones. The Shelbyville test also demonstrated this fact, hence there is no objection to grading seed from this standpoint.

DISCUSSION

Throughout all the various phases of these investigations the economic importance of grading sweet-corn seed for canning purposes was kept in mind. Each experiment demonstrates the difference in rate of growth and time of maturity between the plants from large and small kernels. The stalks from large seed have in all cases been more rapid in growth and more even in maturity than those from small seed. They have also been more resistant to adverse weather conditions and have produced larger yields. For the sake of greater uniformity in date of maturity, it would seem wise to plant the large and small kernels in separate parts of the field, so that the grain may be harvested when it has reached the proper stage without the necessity of picking the whole field twice.

SUMMARY

The large and small kernels of sweet corn germinate and the seedlings come up at about the same time. There is a slight variation in each type, but one has no distinct advantage over the other.

The seedlings from the large kernels are nearly always larger than those from the small ones, and usually remain so. This difference is apparent from the earliest stages.

The large seedlings grow more rapidly and become established in the soil more quickly than the small ones. Consequently, they pass from one stage of development to another in advance of those from the small kernels.

Plants from large kernels reach the tasseling, pollen-shedding, full-silk, and canning stages about five days before those from small kernels. Hence, large seed within a variety tends to produce early maturity and greater uniformity.

Sweet corn for canning purposes should be graded. The large and small kernels should be planted separately.

No difference was found in the size of kernels produced on the ears from large seed and those from small.

There was a larger number of two-eared stalks among those from the large kernels than from the small ones.

More barren and unproductive stalks were found in the plot planted with small kernels than in that planted with large ones.

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THE MAINTENANCE REQUIREMENT OF DRY COWS ¹

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INTRODUCTION

The determination of the maintenance requirements of farm animals is one of the most important of the basic problems of animal industry. The maintenance requirement is the nutriment necessary for sustenance alone, under the living conditions of production. It is therefore the basic requirement to which must be added the direct cost in nutriment of the material product or the labor sought. It is a permanent "overhead" the extent of which must be known before total requirements and costs of production can be satisfactorily determined.

Several methods have been used in the determination of maintenance requirements. Two of these, at least, are of such nature as to yield data of scientific significance.

With man and with carnivora the determination of the body losses during complete fast have yielded relatively accurate measures of the nutriment required to repair tissue waste and to supply the energy needed to keep the body machinery running. With herbivora the problem becomes much more complex, because with these animals the capacity and the anatomy of the digestive tract are such as to render difficult and uncertain the attainment of complete and actual post-resorptive fast.

Fortunately there is another angle of approach to this problem, namely, that proposed by Rubner in his work on isodynamic replacement, and amplified by Armsby (2)³ for the purpose of determining maintenance requirements. This method compares the effects produced by different amounts of feed, such effects being considered as constituting a linear function of the amount of the feed. The essential correctness of this postulate, within certain limits, has been established by the classic experiments of both Rubner and Armsby.

As an illustration of the method the writers quote from Armsby (2, p. 34):

The addition of 2.1 kilograms of timothy hay, equivalent to 3.575 therms of metabolizable energy, to the basal ration reduced the loss of energy from the

¹ Received for publication Apr. 30, 1925; issued January, 1926.

² The experiments on which this paper is based were planned by H. P. Armsby. Responsibility for execution of the details rested largely with J. A. Fries. The computations were made, in large part, by W. W. Braman. The writers wish to express their appreciation of the assistance of E. B. Forbes, director of the institute, in the preparation of the data. They are especially indebted to Max Kriss for his assistance in making the intricate computations and handling the animals during the experiments. They also acknowledge the efficient work of C. D. Jeffries, W. J. Sweeney, R. M. Meredith, Raymond Peterson, and others, who at various times gave their services for the success of the involved and exacting experiments.

³ Reference is made by number (italic) to "Literature cited," p. 1081.

body of the animal by 2.020 therms. [The basal ration supplied 5.687 therms of metabolizable energy and the losses of body protein and fat were equivalent to 2.377 therms per day.] Evidently, then, to have reduced it by 2.377 therms, that is, to zero, would have required the addition of $2.1 \times \frac{2.377}{2.020} = 2.471$ kg. of the hay, equivalent to 4.207 therms of metabolizable energy. The total maintenance ration of this particular feeding stuff, then, would have been the basal ration plus this amount, or 5,670 kg. of the hay, equivalent to 9.894 therms of metabolizable energy.

It will be noted that the above data are expressed in terms of metabolizable energy. So far as the method is concerned it is immaterial whether results are expressed in terms of metabolizable or of net energy but, for reasons which are set forth more fully in the discussion to follow, net-energy values have been used throughout this study.

As to the variability of the maintenance requirements of farm animals, it was expected that one species would differ from another, and that one individual would differ from another of the same species, and the writers found it so. Size, weight, temperament, variations of body temperature as between individuals and from day to day in the same individual, digestive activity, sex, physical condition, age, variation in the time spent lying as compared with the time spent standing—all these, theoretically at least, have a definite bearing on the maintenance requirement. External conditions, such as thermal environment, seasonal variation (10, p. 33), the presence of attendants, and annoyance caused by insects, also have bearing, not only on the maintenance requirement, but also on the accuracy of the determination of it.

A consideration of these many factors which contribute to the maintenance requirement indicates the desirability of directly determining this requirement of an experimental subject, in research on problems of production, rather than applying average figures for maintenance.

In the experiments of this series, the general plan was to determine the maintenance requirement of each individual and to apply these data in the determination of the net-energy values of feeds for milk production and for body increase with this same animal. In a number of instances the writers were unable to carry out this plan and were compelled to make use of average figures.

A perusal of the details of the experiment shows that the heat production and the gaseous excretion were determined on only two days, the ninth and tenth, of the experimental periods. It has been assumed that these two days are representative of the period. That this assumption may introduce an error into the values is undeniable, but that it is not seriously disqualifying is shown by the uniformity of similar data recorded in the work of Armsby and Fries (1), and by the agreement of results from the one experimental day with those from the other.

It will also be noted that these present experiments represent the 24-hour metabolism with normal activity as to standing and lying, and therefore should not be considered as representative of complete repose.

The procedure and the general plan of experimentation have been fully outlined in a previous paper (4).

PLAN OF THE INVESTIGATION

The general routine of these experiments with cows was the same as in the previous work with steers. The experimental ration was fed during a definite period of not less than three weeks. This comprised a preliminary feeding period of about 11 days, and an experimental or "digestion" period of 10 days during which the visible excreta were collected. During the ninth and tenth days of the digestion period the animal was kept in the respiration calorimeter to permit of measurement of the water vapor, carbon dioxide, methane, and heat emission.

PROGRAM OF EXPERIMENTS

Attention is called to the details of Table I. Four different animals were used, the experiments extending over an interval of three years. The four cows used were Nos. 874, 885, 886, and 887.

TABLE I.—*Periods and rations*

Experiment No.	Cow No.	Pe- riod	Preliminary feeding	Calorimeter days	Excreta collected	Daily rations		Feed refused (aver- age per day)
						Grain	Al- falfa hay	
						<i>Kg.</i>	<i>Kg.</i>	<i>Kg.</i>
221D-1919-20.	885	I	Dec. 2-Dec. 30	Jan. 7-Jan. 8	Dec. 30-Jan. 9	2.668	1.779	0.252
	885	II	Jan. 15-Jan. 27	Feb. 4-Feb. 5	Jan. 27-Feb. 6	4.200	2.800	0.728
	885	III	Feb. 8-Mar. 2	Mar. 10-Mar. 11	Mar. 2-Mar. 12	2.668	1.779	0.009
	886	I	Dec. 2-Dec. 9	Dec. 16-Dec. 17	Dec. 9-Dec. 19	2.614	1.745	None.
	886	II	Dec. 25-Jan. 13	Jan. 21-Jan. 22	Jan. 13-Jan. 23	4.200	2.800	0.020
	886	III	Jan. 25-Feb. 17	Feb. 25-Feb. 26	Feb. 17-Feb. 27	2.614	1.745	None.
221E-1921-....	885	I	Jan. 22-Feb. 1	Feb. 9-Feb. 10	Feb. 2-Feb. 11	3.760	2.540	0.946
	885	II	Feb. 12-Feb. 22	Mar. 2-Mar. 3	Feb. 23-Mar. 4	2.360	1.580	0.009
221F-1921-22.	874	I	Dec. 3-Dec. 13	Dec. 21-Dec. 22	Dec. 14-Dec. 23	4.100	2.733	None.
	874	II	Jan. 7-Jan. 17	Jan. 25-Jan. 26	Jan. 18-Jan. 27	2.744	1.830	None.
	887	I	Feb. 4-Feb. 14	Feb. 22-Feb. 23	Feb. 15-Feb. 24	3.696	2.464	None.
	887	II	Feb. 25-Mar. 7	Mar. 15-Mar. 16	Mar. 8-Mar. 17	2.474	1.650	None.

Cow 885 was dry during two successive years, having failed to get with calf during the second year, and was used twice in connection with the maintenance experiments. Cows 874, 886, and 887 were used during only one year in this study of maintenance requirements. All these animals were used in experiments on milk production, the results of which will be reported in a separate paper.

ANIMALS

The subjects used were either purebred or grade Jerseys of average size and quiet disposition.

Cow 885 was a purebred Jersey, registry No. 333236, dropped March 27, 1914. She gave birth to her first calf September 15, 1917, and was not bred again prior to the beginning of this study.

Cow 886 was a purebred Jersey, registry No. 333886, dropped July 13, 1914. She gave birth to her first calf October 10, 1917, and was bred again just prior to the beginning of this study.

Cow 874, of unknown breeding, was dropped May 5, 1916. She was typically Jersey in conformation and color, was fresh twice, the

second time on July 27, 1920, and was not bred again prior to the beginning of this study.

Cow 887, a purebred Jersey, was dropped September 5, 1917. She was fresh twice, January 11, 1920, and March 11, 1921, and was not bred again prior to the beginning of this study.

RATIONS

The same feeding stuffs, mixed in the same proportions, were fed throughout the experiment. The quantity fed each animal was so adjusted as to maintain approximately constant live weight in experiments 221D-885-I and III, experiments 221D-886-I and III, experiment 221E-885-II, experiment 221F-874-II, and experiment 221F-887-II. In the other periods, 221D-885-II, 221D-886-II, 221E-885-I, 221F-874-I, and 221F-887-I the feed was increased so that the animals gained materially in body weight.

The grain mixture fed was composed of 30 parts wheat bran, 30 parts ground oats, 30 parts corn meal, and 10 parts old process linseed meal, all of good quality. The roughage was good-quality alfalfa, grown in Colorado, nicely cured, of uniform green color, containing a normal proportion of leaves.

The details of methods of handling, weighing, sampling and analysis of feeds, feces and urine have been published (4, p. 8-9).

For a description of the respiration calorimeter and the minutiae of methods for its operation, reference is made to the publications of Armsby and Fries.

DIGESTIBILITY OF THE RATION

Owing to the impossibility of obtaining attendants, it was impracticable to collect the dung and urine separately during the digestion period. Hence, in order to get an approximate value for the apparent digestibility of the ration, the dung and urine were collected separately on one day of the experimental period by members of the institute staff. On the other days of the digestion period the dung and urine were collected together, by means of a conducting apron devised by one of the writers, Fries.

Assuming the relative constancy of composition of the dry matter of the dung under the conditions of the experiment, it is possible, by making use of the percentage of crude fiber in the dung and urine mixture, and in the dung alone, to approximate the apparent digestibility of the various constituents of the feed. The details of this computation will be more fully discussed later.

LIVE WEIGHT

The animals were weighed daily after the morning feeding and before watering. During the 48 hours spent in the respiration calorimeter live weights could not be taken. The animals, however, were weighed on entering the chamber and again on leaving it. For the average weight of the animals during the experimental periods the weights on the 10 days immediately preceding the calorimeter days, including the last two days of the preliminary period, have been used (Table II).

TABLE II.—Average daily live weights of experimental subjects

Experiment and cow Nos.	Period I	Period II	Period III
	Kg.	Kg.	Kg.
221D-885.....	415. 22	429. 74	^a 426. 36
221D-886.....	399. 78	424. 41	420. 19
221E-885.....	443. 12	434. 36	-----
221F-874.....	428. 80	415. 58	-----
221F-887.....	335. 21	320. 49	-----

^a Only 9 days in Period III, 221D-885.

DETAILS OF ANALYSES

The customary feeding-stuff analyses were made on the alfalfa hay, grain mixture, and excreta. These data, together with the heat of combustion, have been computed to a dry-matter basis, and are presented in Tables III, IV, and V.

TABLE III.—Composition of the dry matter of the feed

Substance	Experiment No.	Cow and period No.	Ash	Protein ^a	Non-protein ^b	Crude fiber	Nitrogen-free extract	Ether extract	Total nitrogen	Protein nitrogen	Carbon	Energy per gram
			Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Calories
Alfalfa hay..	221D	885-I	7. 85	11. 09	2. 36	37. 86	36. 80	2. 03	2. 28	1. 77	46. 08	4. 53943
		886-I										
		885-II										
	221E	886-II	8. 38	12. 11	2. 42	34. 18	40. 84	2. 07	2. 45	1. 94	46. 12	4. 52408
		885-III										
		886-III										
Grain mixture.	221F	885-I-II	8. 44	11. 61	2. 49	34. 75	40. 75	1. 97	2. 39	1. 86	46. 05	4. 57295
		885-I-II										
		874-I-II										
	221D	887-I-II	8. 03	11. 52	2. 18	36. 93	39. 70	1. 64	2. 31	1. 84	46. 55	4. 54493
		885-I										
		886-I										
Refused feed	221E	885-II	4. 69	13. 13	2. 14	9. 02	66. 12	4. 90	2. 70	2. 24	46. 25	4. 62553
		886-II										
		885-III										
	221F	886-III	4. 57	12. 79	2. 21	10. 62	65. 05	4. 76	2. 65	2. 18	46. 26	4. 62276
		885-I-II										
		874-I-II										

^a True protein; A. O. A. C. method.

^b Nonprotein, N×4.7.

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TABLE IV.—Composition of the dry matter of the dung alone

Experiment, animal, and period Nos.	Ash	Pro- tein ^a	Non- pro- tein ^b	Crude fiber	Nitro- gen- free extract	Ether extract	Total nitrogen		Carbon		Energy per gram
							From fresh sub- stance ^c	From air-dry sub- stance	From fresh sub- stance	From air-dry sub- stance	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Calories
221D-885-I.....	11.88	10.23	1.95	37.52	35.95	2.48	2.05	2.00	(^d)	46.67	4.66491
II.....	12.40	11.53	2.15	36.34	35.30	2.29	2.30	2.09	(^e)	46.69	4.68633
III.....	12.27	10.61	2.05	37.71	34.96	2.39	2.13	2.07	(^e)	46.59	4.68649
221D-886-I.....	12.42	9.72	1.98	38.48	34.78	2.63	1.98	1.89	(^e)	46.51	4.62183
II.....	12.89	10.71	2.54	35.05	36.26	2.55	2.25	2.11	(^e)	46.10	4.56661
III.....	12.41	11.28	2.03	36.93	34.86	2.51	2.24	2.22	(^e)	46.28	4.63401
221E-885-I.....	11.26	10.12	1.50	38.04	36.94	2.15	1.94	1.80	(^e)	46.84	4.69723
II.....	11.78	11.56	0.91	37.11	36.58	2.06	2.04	1.97	(^e)	46.28	4.63002
221F-874-I.....	11.28	10.86	1.66	34.79	39.03	2.37	2.09	2.00	48.00	46.95	4.70966
II.....	12.19	10.16	1.69	35.42	38.16	2.39	1.93	1.99	47.23	46.02	4.69322
221F-887-I.....	11.33	10.24	3.29	31.90	40.99	2.25	2.34	1.97	49.20	45.82	4.69662
II.....	11.06	10.21	2.51	38.67	35.40	2.16	2.17	1.93	48.05	46.16	4.68011

^a True protein; A. O. A. C. method.^b Nonprotein, N×4.7.^c By König's method.^d No determinations of carbon in the fresh substance were made prior to experiment 221F.^e By direct combustion of the fresh material.

TABLE V.—Composition of the dry matter of the dung and urine mixture

Experiment, animal, and period Nos.	Ash	Pro- tein ^a	Non- pro- tein ^b	Crude fiber	Nitro- gen- free extract	Ether extract	Total nitrogen		Carbon		Energy per gram
							From fresh sub- stance ^c	From air-dry sub- stance	From fresh sub- stance	From air-dry sub- stance	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Calories
221D-885-I.....	18.46	8.98	26.28	30.46	13.41	2.42	7.25	3.21	(^e)	43.24	4.39446
II.....	17.24	9.36	23.30	29.97	17.64	2.49	6.66	2.88	(^d)	43.72	4.41033
III.....	19.22	9.21	24.28	28.25	16.30	2.74	6.85	3.00	(^d)	42.89	4.33180
221D-886-I.....	17.43	8.84	22.38	29.81	18.54	3.00	6.38	2.81	(^d)	43.22	4.34790
II.....	17.89	9.46	24.32	28.81	16.95	2.58	6.86	3.10	(^d)	43.17	4.34558
III.....	17.83	9.81	22.80	29.58	17.51	2.48	6.60	2.63	(^d)	43.58	4.35050
221E-885-I.....	17.03	9.98	13.33	29.07	27.98	2.61	5.97	2.78	(^d)	44.01	4.42019
II.....	18.11	9.63	24.15	29.15	16.72	2.25	6.89	2.93	46.58	43.67	4.32726
221F-874-I.....	15.30	9.02	23.12	29.80	20.44	2.32	6.36	2.85	47.03	43.80	4.42538
II.....	16.48	8.64	23.10	28.53	20.72	2.52	6.30	2.98	47.53	43.65	4.38052
221F-887-I.....	16.85	8.82	25.05	29.85	17.33	2.11	6.74	2.40	47.95	44.21	4.45052
II.....	17.26	9.09	26.79	28.19	16.57	2.10	7.15	2.59	47.63	43.89	4.39412

^a True protein, A. O. A. C. method.^b Nonprotein, N×4.7.^c No determinations of carbon in the fresh substance were made prior to experiment 221E.^d By direct combustion of the fresh materials.

The analyses of the dung alone represent the composition of the dung as collected separately on a single day of each period, and are used only to obtain an approximation of the apparent digestibility. For purposes of comparison, the total nitrogen was determined on the fresh material by König's method, and also on the sample after air drying by the Kjeldahl method. The data show clearly that nitrogen was lost during the drying of the sample. Beginning with experiment 221F the carbon in the fresh material also was determined by direct combustion as well as in the air-dry sample. The loss of carbon shown is of relatively greater consequence in subsequent computations than is the loss of nitrogen, and may not be neglected without introducing material error.

The results obtained by the analysis of the dung and urine mixture as shown in Table V are used to obtain the balance of matter, and are the basis of all computations except those relating to the apparent digestibility. Here again the loss of nitrogen and carbon during drying is evident, this loss apparently being due to fermentation.

THE APPARENT DIGESTIBILITY OF THE RATION

In order that the method by which the writers have arrived at an approximation of the apparent digestibility of the various constituents of the ration may be clear, the computation for experiment 221D-885-I has been included in detail in Table VI. The method is based primarily on the assumption that the percentage composition of the dry matter of the dung is approximately constant for a given ration, an hypothesis which seems to be justified by an examination of data previously published from the institute. In connection with the second part of the computation (the fresh weight of the dung alone) it has been necessary to assume the percentage dry matter of the dung alone to be a constant on a given ration. There is a measure of uncertainty in this assumption. However, since this factor affects only the urine, no great error results from this procedure.

TABLE VI.—Example of computation of apparent digestibility (221D-885-I)

Average daily feeds and excreta; fresh weights; 10-day period		Dry matter		
		Kg. per day	Per cent	Grams per day
Alfalfa hay	-----	1.779	89.26	1,588
Mixed grain	-----	2.668	86.28	2,302
Salt	-----	.030	98.77	30
Refused feed	-----	.252	88.25	223
Dung and urine	-----	9.415	14.29	1,346
Apron scrapings	-----	.020	14.29	3
Computed dung alone	-----	4.574	-----	1,095
Computed urine alone	-----	4.862	-----	254
Spilled urine	-----	.007	-----	-----
Total urine	-----	4.869	-----	254
Totals:				
Dung and urine mixture	-----	9.443	-----	-----
Dung alone	-----	4.574	-----	-----
Urine alone	-----	4.869	-----	-----

CONSTITUENTS OF FEEDS AND EXCRETA

	Dry matter	Organic matter	Protein ^a	Non-protein ^b	Crude fiber	Nitro-gen-free extract	Ether extract	Total nitrogen	Carbon	Energy
	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Cals.
Salt	29.6									
Alfalfa hay	1,588.0	1,463.3	176.2	37.5	601.1	616.2	32.2	36.2	731.7	7,208.6
Mixed grain	2,302.0	2,194.0	302.2	49.3	207.7	1,522.1	112.8	62.1	1,064.6	10,648.0
Refused feed	222.6	208.3	32.7	6.2	24.1	136.4	8.9	6.6	101.5	1,012.7
Total eaten	3,697.0	3,449.0	445.7	80.6	784.7	2,001.9	136.1	91.7	1,694.8	16,843.9
Dung	1,095.0	964.9	112.0	21.4	410.8	393.6	27.2	22.5	500.5	4,949.0
Total digested	2,602.0	2,484.1	333.7	59.2	373.9	1,608.3	108.9	69.2	1,194.3	11,894.9
Per cent digested	70	72	75	73	48	80	80	75	70	71

^a True protein; A. O. A. C. method.
^b Nonprotein, N×4.7.
^c Corrected for loss on drying (see Table VIII for method and factors used):
Computation of dung in mixture:
Dry matter of dung and urine mixture : X :: per cent crude fiber in dung alone : per cent crude fiber in dung and urine mixture.
$$\frac{1,349 \times 30.46}{37.52} = 1,095.0 \text{ grams.}$$

Computation of fresh weight of dung:
Dry matter of dung alone (computed) : X :: per cent of dry matter of dung alone : 100
$$\frac{1,095.0}{23.94} \times 100 = 4,574 \text{ grams.}$$

In Table VII the coefficients of apparent digestibility, computed as explained above, have been collected. The averages are arithmetic, not weighted, means.

TABLE VII.—Coefficients of apparent digestibility (feed minus feces)

Experiment, animal, and period Nos.	Dry matter	Organic matter	Pro- tein	Non- pro- tein *	Crude fiber	Nitro- gen free- extract	Ether extract	Total nitro- gen	Carbon	Organic matter as energy
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
221D-885-I.....	70	72	75	73	48	80	80	75	70	71
II.....	70	72	72	72	49	81	82	73	69	69
III.....	74	75	77	77	50	84	82	78	73	73
Average.....	71	73	75	74	49	82	81	75	71	71
221D-886-I.....	72	74	78	75	48	82	80	78	71	71
II.....	70	72	75	67	48	80	79	74	70	70
III.....	72	73	74	75	47	82	80	75	71	71
Average.....	71	73	76	72	48	81	80	76	71	71
221E-885-I.....	73	74	80	68	49	82	83	79	72	72
II.....	73	74	76	82	45	82	84	78	72	72
Average.....	73	74	78	75	47	82	84	79	72	72
221F-874-I.....	68	70	71	73	46	78	79	73	67	67
II.....	70	72	74	74	47	79	80	75	69	69
Average.....	69	71	73	74	47	79	80	74	68	68
221F-887-I.....	68	69	73	46	46	77	79	69	65	67
II.....	74	75	78	67	48	84	84	77	73	74
Average.....	71	72	76	57	47	81	82	73	69	71

* Nonprotein, N×4.7.

THE LOSS OF NITROGEN AND CARBON ON DRYING SAMPLES OF EXCRETA PRIOR TO GRINDING

The loss of nitrogen and carbon in drying during the preparation of the samples for analysis has been a recognized source of error throughout the entire series of investigations with the respiration calorimeter. In the experiments with steers the feces and the urine were handled separately, and the loss on drying the feces was found to be small, and was disregarded; while the energy was corrected for the urinary loss on the basis of Rubner's figure of 5.45 Cal. per gram of nitrogen lost on drying. The data for the first experiment with cows, 221A, were also handled in this way, as the feces and urine were collected separately.

In all experiments with these cows subsequent to 221A it was necessary to collect the feces and urine as a mixture, and it was soon recognized that the physical state of this mixture, particularly its moisture content, might be important in relation to fermentation during preparation for analysis. The decomposition of these mixed samples during drying was greater than that of either the dung or the urine alone, and especially significant was it that more carbon was lost than could be accounted for as originating in the nitrogenous compounds of the urine in the mixture. There is a possibility, however, that the loss of nitrogen observed may be somewhat less than the true loss, since fixation of nitrogen has been shown to occur in drying feces (8).

This loss of carbon unaccompanied by nitrogen may result from two sources, viz, from a continuation of the intestinal fermentation after the feces have been voided, and from the breaking up of the nonnitrogenous compounds in the urine. That both processes contribute to this excess of carbon dioxide seems probable. However, the present state of the writers' knowledge of the compounds involved in the decomposition of the urine is insufficient to serve as the basis for the correction of its energy, and this has not been attempted. Neither has the energy of the urine been corrected for the free ammonia and free carbon dioxide present.

When the sources of the carbon dioxide lost during the drying of the mixed feces and urine, in excess of that coming from the nitrogenous compounds of the urine, are considered, to wit, the crude fiber and the nitrogen-free extract of the dung, the writers are impressed with the possibility of extensive error. Soon after the mixture is placed in the drying closet a thick scum forms over the surface, this scum preventing rapid drying and at the same time maintaining in the interior of the mass conditions favorable for the continuance of digestive cleavage and for the development of bacteria with the resultant formation of methane and carbon dioxide. This condition was not recognized early enough in this series of experiments to have permitted of direct determinations of the amounts of the gases lost. However, in all experiments following 221E the total carbon was determined by direct combustion of the fresh material, and this, in conjunction with the total nitrogen as determined in the fresh substance by König's method, furnishes sufficient data to make possible a computation of the energy loss due to the aforementioned factors.

It is apparent, then, that the carbon lost on drying the mixed feces and urine must be divided into three parts, in so far as its relation to the corresponding energy loss is concerned: (1) That portion which is combined with nitrogen and is accounted for through the use of Rubner's factor of 5.45 Cal. per gram of nitrogen lost on drying; (2) that portion which is present in solution as part of the system $\text{NH}_3 \cdot \text{CO}_2 \cdot \text{H}_2\text{O}$ (this is to some extent compensated for by the fact that a portion of the NH_3 present in this system has been included in the total nitrogen lost on drying, its carbon equivalent thus being taken into account, as will later appear); (3) the residuum of carbon considered as originating in the fermentation of the nonnitrogenous material.

Since this fermentation simulates intestinal fermentation, not only carbon dioxide but also methane is produced. This latter the writers have been unable to determine quantitatively. However, in view of the relatively small difference between the heats of combustion of crude fiber and nitrogen-free extract, the writers feel justified in computing the amount of energy lost through this external fermentation from the carbon dioxide produced and the energy equivalent of starch, as representing nitrogen-free extract generally.

Since in experiment 221D the carbon was not determined in the fresh dung and urine mixture, it was necessary to compute the correction for this experiment on the basis of average figures. The data upon which this computation is based are indicated in Table VIII.

TABLE VIII.—Carbon and nitrogen lost per day through the drying of the dung and urine mixture

Experiment, animal, and period Nos.	Dry matter ^a	Water	Nitrogen lost	Carbon lost			Energy equivalents		Energy of air-dry material	Energy of air-dry material, corrected—	
				Total	Combined with nitrogen	From carbohydrate	N× 5.45	Starch C× 9.4		For nitrogen	For nitrogen and carbohydrate fermentation
	Grams	Grams	Grams	Grams	Grams	Grams	Cals.	Cals.	Cals.	Cals.	Cals.
221D-885-I-----	1,349.1	8,093.5	^b 54.5	^c 62.7	23.4	^c 39.3	297.0	^c 369.4	5,928.6	6,225.6	6,595.0
II-----	1,982.9	14,682.9	^b 74.8	^c 86.0	32.1	^c 53.9	407.7	^c 506.7	8,745.7	9,153.4	9,660.1
III-----	1,355.0	9,097.9	^b 52.3	^c 60.1	22.4	^c 37.7	285.0	^c 354.4	5,869.3	6,154.3	6,508.7
221D-886-I-----	1,396.4	7,901.2	^b 49.9	^c 57.4	21.4	^c 36.0	272.0	^c 338.4	6,071.4	6,343.4	6,681.8
II-----	2,200.3	13,434.2	^b 82.8	^c 95.2	35.5	^c 59.7	451.3	^c 561.2	9,557.2	10,008.5	10,569.7
III-----	1,347.8	9,161.5	^b 53.5	^c 61.5	23.0	^c 38.5	291.6	^c 361.9	5,863.6	6,155.2	6,517.1
221E-885-I-----	1,672.8	10,180.5	^b 53.2	^c 61.2	22.8	^c 38.4	289.9	^c 361.0	7,394.1	7,684.0	8,045.0
II-----	1,218.3	7,799.5	48.1	69.3	20.6	48.7	262.1	457.8	5,271.9	5,534.0	5,991.8
221F-874-I-----	2,225.8	11,144.2	78.3	71.9	33.6	38.3	426.7	360.0	9,850.0	10,276.7	10,636.7
II-----	1,516.0	7,512.4	50.3	58.8	21.6	37.2	274.1	349.7	6,641.5	6,915.6	7,265.3
221F-887-I-----	1,887.4	11,543.3	82.0	70.7	35.2	35.5	446.9	333.7	8,400.1	8,847.0	9,180.7
II-----	1,296.5	7,626.2	59.2	48.5	25.4	23.1	322.6	217.1	5,697.0	6,019.6	6,236.7
<i>Cows in milk</i>											
221E-886-I-----	-----	-----	^b 79.9	^c 91.9	34.3	^c 57.6	435.5	^c 541.4	-----	-----	-----
II-----	-----	-----	75.9	69.8	32.6	37.2	413.7	349.7	-----	-----	-----
221E-874-I-----	-----	-----	^b 65.2	^c 75.0	28.0	^c 47.0	355.3	441.8	-----	-----	-----
II-----	-----	-----	^b 60.0	^c 69.0	25.7	^c 43.3	327.0	^c 407.0	-----	-----	-----
221F-886-I-----	-----	-----	83.9	119.6	36.0	83.6	457.3	785.8	-----	-----	-----
II-----	-----	-----	96.8	124.7	41.5	83.2	527.6	782.1	-----	-----	-----
221G-887-I-----	-----	-----	75.7	108.3	32.5	75.8	412.6	712.5	-----	-----	-----
II-----	-----	-----	58.5	82.2	25.1	57.1	318.9	536.7	-----	-----	-----
III-----	-----	-----	61.4	79.3	26.3	53.0	334.6	498.2	-----	-----	-----
IV-----	-----	-----	87.3	83.5	37.5	46.0	475.8	432.4	-----	-----	-----

^a Not corrected for loss on drying.
^b Not included in average used to obtain data for carbon correction.
^c Computed factors:
1 gram C \approx 2.25 grams starch.
1 gram N lost \approx 1.15 grams carbon lost.
1 gram carbon lost \approx 0.627 gram carbon from fermentation of carbohydrates.
1 gram N \approx 3.43 grams ammonium carbonate.
1 gram carbon of carbohydrate fermented (starch) \approx 9.4. Cal.
Ratio of carbon to nitrogen in urea or in ammonium carbonate $\frac{C}{N}$ = 0.429.

In Table IX similar data for the loss during the drying of the dung alone have been compiled for reference. The dung alone is used only in arriving at approximate figures for the apparent digestibility, and the data available for the loss on drying are few and widely variable. Therefore, it has seemed best not to apply the correction to the carbon and energy of the dung alone in this particular series of experiments, but rather to place the data on record in order to call attention to the fact that the loss of carbon, and its equivalent energy, during the preparation of samples of feces for analysis, is a material and a variable factor which must be taken into account in exact experimentation involving digestibility.

TABLE IX.—Carbon and nitrogen lost per day during the drying of dung alone

Experiment, animal, and period Nos.	Dry mat- ter ^a	Water	Nitro- gen lost	Carbon lost			Energy equivalents		Energy of air-dry ma- terial	Energy of air-dry material, corrected—	
				Total	Com- bined with nitro- gen. N× 0.429	From car- bohy- drate	N× 5.45	Starch C×9.4		For nitro- gen	For nitro- gen and carbo- hydrate fermen- tation
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-885-I-----	1,095.0	3,478.7	0.6	(b)	0.3	c 9.8	3.3	92.1	5,108.1	5,111.4	5,203.5
II-----	1,635.7	8,346.0	3.4	(b)	1.5	c 48.8	18.5	458.7	7,665.4	7,683.9	8,142.6
III-----	1,014.2	4,103.1	.6	(b)	.3	c 9.8	3.3	92.1	4,753.0	4,756.3	4,848.4
221D-886-I-----	1,081.7	4,035.1	1.0	(b)	.4	c 13.0	5.5	122.2	4,999.9	5,005.4	5,127.6
II-----	1,808.5	7,541.1	2.5	(b)	1.1	c 35.8	13.6	336.5	8,258.7	8,272.3	8,608.3
III-----	1,079.7	4,776.8	.1	(b)	.0	c .0	.5	.0	5,003.3		
221E-885-I-----	1,279.8	4,840.0	1.8	(b)	.8	c 26.0	9.8	244.4	6,011.5	6,021.3	6,265.7
II-----	955.9	3,974.6	.7	(b)	.3	c 9.8	3.8	92.1	4,425.8	4,429.6	4,521.7
221F-874-I-----	1,906.6	7,201.9	1.8	c 19.9	c .8	c 19.1	9.8	179.5	8,979.4	8,989.2	9,168.7
II-----	1,221.7	3,848.0	None.	c 14.7	.0	c 14.7	.0	138.2	5,733.7		5,871.9
221F-887-I-----	1,766.1	7,153.5	6.5	c 59.6	2.8	c 56.8	35.4	533.9	8,294.7	8,330.1	8,864.0
II-----	945.4	3,441.0	2.3	c 18.0	1.0	c 17.0	12.5	159.8	4,424.6	4,437.1	4,596.9
<i>Cows in milk</i>											
221E-886-I-----			1.3	(b)	.6	c 16.3	2.8	-----			
II-----			2.2	(b)	.9	c 29.3	4.9	-----			
221E-874-I-----			2.3	(b)	1.0	c 29.3	4.9	-----			
II-----			1.7	(b)	.7	c 22.8	3.8	-----			
221F-886-I-----			6.6	52.7	2.6	50.1	14.2	-----			
II-----			9.9	75.6	4.0	71.6	21.8	-----			
221G-887-I-----			3.4	30.2	1.4	28.9	7.6	-----			
II-----			1.4	13.5	.6	12.9	3.3	-----			
III-----			2.8	22.1	1.1	21.0	6.0	-----			
IV-----			2.3	78.1	.9	77.2	4.9	-----			

^a Not corrected for loss on drying.
^b Not determined.
^c Computed; based on average figures.

In this study the uncorrected dry matter must be used as the basis for the computations, because the analyses were made on the dried substance which had sustained the losses above referred to. Therefore, in the case of the dung and urine mixture, the appropriate corrections have been applied to the individual constituents affected.

PRODUCTION OF EPIDERMAL TISSUE

The cows were not clipped, as were the steers in the earlier experiments. The animals were thoroughly brushed, however, before being placed in the respiration calorimeter, and again on leaving it. The material obtained by this second brushing has been taken as representing the growth of hair, epithelium, etc., during the time spent in the calorimeter. Owing to the inadvertent destruction of a sample, the data for experiment 221D-886-I are incomplete. The average for Periods II and III was therefore used. The data for this loss are included in Table X.

TABLE X.—Loss as hair and scurf per day

Experiment, animal, and period Nos.	Dry matter	Nitrogen	Carbon	Energy
	Grams	Grams	Grams	Calories
221D-885-I.....	13.9	1.3	6.1	65.9
II.....	16.7	1.9	7.5	81.5
III.....	14.9	1.8	6.5	71.5
221D-886-I.....	^a 12.1	^a 1.2	^a 5.4	^a 59.1
II.....	14.1	1.3	6.2	67.0
III.....	10.1	1.1	4.7	51.1
221E-885-I.....	15.2	1.2	6.0	64.2
II.....	11.0	1.2	4.6	50.0
221F-874-I.....	11.6	1.1	5.1	56.0
II.....	11.8	1.1	5.2	57.2
221F-887-I.....	13.3	1.4	6.2	68.1
II.....	11.8	1.2	5.0	55.9

^a Average of Periods II and III, cow 886.

THE GASEOUS EXCRETA

With the animals in the respiration calorimeter the carbon dioxide, methane, and water vapor excreted were determined by methods which have been described in previous publications of the institute. The data so obtained for this series of experiments are presented in Table XI. It will be noted that the ratio of carbon to hydrogen in the combustible gases excreted by these cows was quite variable. This was in part due to the difficulty experienced in the determination of the hydrogen. However, there was on the average an excess of hydrogen to the amount theoretically required to unite with the carbon to form methane. This finding is borne out by subsequent unpublished experiments, but the variability of the results, due to imperfections in the hydrogen estimation, is such as to render impossible definite interpretation of this apparent excess of hydrogen. In experiment 221D-886-I an accident to the apparatus rendered the completion of the two-day period impossible, and the data recorded are for one day only.

TABLE XI.—Carbon, hydrogen, and water vapor in daily gaseous excreta ^a

Experiment, animal, and period Nos.	Water vapor	Carbon dioxide	Carbon ^b	Hydrocarbons			Ratio of carbon to hydrogen ($\frac{C}{H}$)
				Hydrogen	Carbon	Methane ^c	
	Grams	Grams	Grams	Grams	Grams	Grams	
221D-885-I.....	4,866.7	3,360.9	916.5	26.0	70.2	93.7	2.700
II.....	6,544.4	4,067.6	1,109.2	31.3	101.3	135.4	3.236
III.....	5,130.5	3,695.8	1,007.8	29.7	88.2	117.8	2.970
221D-886-I ^d	4,213.3	3,440.0	938.1	31.2	81.7	109.2	2.619
II.....	6,932.0	4,720.7	1,287.3	39.9	115.9	154.9	2.905
III.....	3,726.8	3,409.0	929.6	29.1	85.1	113.8	2.924
221E-885-I.....	6,568.9	3,940.0	1,074.4	41.1	91.0	121.6	2.214
II.....	4,886.3	3,515.5	958.7	31.6	78.3	104.6	2.478
221F-874 I.....	5,851.7	4,881.6	1,331.2	41.4	118.1	157.8	2.853
II.....	4,034.8	3,808.8	1,038.7	29.4	86.0	114.9	2.925
221F-887-I.....	5,309.5	4,549.1	1,240.5	38.1	114.2	152.6	2.997
II.....	3,256.9	3,521.8	960.4	28.1	80.7	107.9	2.872

^a Corrected for man entering chamber to make adjustments.
^b Computed from CO₂, grams CO₂ × 0.2727.
^c Computed from carbon in hydrocarbons.
^d One-day experiment.

THE BALANCE OF MATTER

From the data in the preceding tables the balance of matter per day and head has been computed as recorded in Table XII. The balance of matter represents the average for the 10-day period; the balance of water that of the 2-day calorimeter test. In determining the water balance, on the 2 respiration-calorimeter days, the daily portions of the feeds as previously weighed were reweighed shortly before being introduced into the chamber, any difference being considered as representing a gain or loss of water. Also special samples were taken from the excreta voided in the calorimeter for the determination of dry matter. The nitrogen outgo was determined by analysis of the fresh, undried excreta, and the carbon has been corrected for the loss on drying. The organic hydrogen oxidized in the body has been ignored as being of a magnitude of no material importance in the water balance. The factors and the general method involved are those customarily used, and, as in previous experiments, the schematic body is assumed to consist of two variables, protein and fat, and a constant, glycogen.

TABLE XII.—The gain or loss of water, protein, and fat per day and per head

Experiment, animal, and period Nos.	Water	Protein	Fat
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
221D-885-I.....	-4,063.0	-45.0	+103.9
II.....	-3,458.4	+28.2	+447.3
III.....	-1,531.5	+16.2	+28.9
221D-886-I.....	-9,284.7	+36.0	+72.9
II.....	-6,586.4	+25.2	+446.9
III.....	-5,597.2	+33.6	+74.1
221E-885-I.....	-3,616.3	+103.8	+214.1
II.....	-1,882.5	+19.8	-28.2
221F-887-I.....	-6,219.7	+25.8	+302.3
II.....	-2,393.2	-29.4	+42.9
221F-874-I.....	-3,721.5	+16.8	+358.4
II.....	-1,502.7	+6.0	+13.2

The animals, without exception, drank materially less water per day while in the respiration calorimeter than during the "digestion" period. This indicates that they were varying from normal to some extent while in the calorimeter. However, the actual influence of this variation may readily be taken into account in the computation of the heat production, of the metabolizable energy, and of net-energy values.

The data indicate a loss of protein, coincident with a gain of fat, in experiments 221D-885-I and 221F-887-II. In experiment 221E-885-II the apparent balances were reversed; protein was gained while fat was lost. The net balances of dry matter in the maintenance periods show that in all cases more feed was given than was required to maintain equilibrium.

In experimental series 221D the third period for each cow was designed to duplicate the first. In the case of cow 885 it is apparent that the utilization of the feed, as measured by the gain or loss of protein and fat, was not the same. This difference may be ascribed in part to the refusal of feed in Period I, which view is substantiated by a comparison with data obtained in the corresponding periods with cow 886 in which the experimental conditions were identical except that there was no refused feed, the data from the two periods

being in close agreement. That the effect of the refused feed is so marked in this particular instance is no doubt due to the fact that the cow went badly off feed just prior to the respiration-calorimeter test.

Table XIII sets forth a correction to be applied to the dry matter of the excreta on account of irregularity of excretion. This is necessary because the balances of nitrogen and carbon are computed on the basis of the 10-day period, the correction rendering the outgo during the calorimeter days representative of the digestion period as a whole.

TABLE XIII.—Correction for irregularity of excretion

Experiment, animal, and period Nos.	Dry matter of the dung and urine mixture (per day)		Correction
	10-day period ^a	Calorimeter day ^a	
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
221D-885-I.....	1,349.1	955.3	+393.8
II.....	1,982.9	1,185.5	+797.4
III.....	1,355.0	996.6	+358.4
221D-886-I.....	1,396.4	1,033.3	+363.1
II.....	2,200.3	1,814.1	+386.2
III.....	1,347.8	1,071.6	+276.2
221E-885-I.....	1,672.8	1,439.5	+233.4
II.....	1,218.3	1,069.5	+148.8
221F-874-I.....	2,494.4	2,254.6	+239.8
II.....	1,688.5	1,434.0	+254.5
221F-887-I.....	2,168.7	1,934.3	+234.4
II.....	1,499.6	1,649.6	-150.0

^a Not corrected for loss on drying.

The respiration calorimeter is not equipped for weighing the animal. The subject is weighed, however, just before entering, and again as it is removed from the chamber. In every period there was a loss in live weight during the calorimetric measurements. This is largely due to the failure of the cows to drink as much water while in the calorimeter as during the 10-day digestion period.

As in previous work of the institute, the heat emission of the animal was determined by direct measurement on two consecutive days of the experimental period. The methods used and a description of the apparatus employed have been covered in detail in the various publications of Armsby and Fries. The heat emission per day—by radiation and conduction and as latent heat of water vapor—is given in Table XIV.

TABLE XIV.—Daily emission and production of heat

Experiment, animal, and period Nos.	Heat emission	Correction for body gain	Heat production
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-885-I.....	8,462.8	-82.5	8,380.3
II.....	9,667.7	-60.3	9,607.4
III.....	8,845.7	-29.0	8,816.7
221D-886-I.....	8,252.2	-193.6	8,058.6
II.....	10,603.8	-129.3	10,474.5
III.....	8,030.0	-115.9	7,914.1
221E-885-I.....	9,791.2	-69.5	9,721.7
II.....	8,773.4	-37.9	8,735.5
221F-874-I.....	11,037.1	-72.9	10,964.2
II.....	9,106.3	-29.5	9,076.8
221F-887-I.....	10,451.1	-123.9	10,327.2
II.....	8,446.7	-51.7	8,395.0

DAILY EMISSION AND PRODUCTION OF HEAT

It is obvious that the heat emission equals the heat production only in case there is neither gain nor loss of matter by the body during the time of measurement. In the experiments herein considered the animals gained or lost varying amounts of fat and protein. Hence, it is necessary to compute the thermal equivalent of these gains or losses in order to arrive at the heat production of the animal. This computation has the effect to bring the body mathematically to its state as at the beginning of the experiment.

In order that this computation may not be confused with the determination of the energy value of the tissue exchange, the data for experiment 221E-885-I are given in detail.

COMPUTATION OF THE CORRECTION FOR THE TEMPERATURE OF THE GAIN BY THE BODY, EXPERIMENT 221E-885-I

Average body temperature, 38.61° C.; calorimeter temperature, 17.86° C.			
Protein-----	+0.1038 kg.×0.3	×20.75=	+ 0.65 Cal.
Fat-----	+0.2141 kg.×0.66	×20.75=	+ 2.93 Cal.
Water-----	-3.6163 kg.×1.0	×20.75=	-75.04 Cal.
Irregular excretion of dry matter---	0.2334	×0.4	×20.75= + 1.93 Cal.
Correction for gain by body-----			-69.53 Cal.

It will be noted that the average body temperature of the animal has been used, ignoring the small daily fluctuations. For the specific heats of protein and fat, Rosenthal's values (9) have been applied. In the experiments with steers variations in the dry matter excreted per day were relatively small, and this item was neglected in computing the thermal effect correction. However, in these experiments the variations were so great as to require correction as shown above.

Since the nitrogen contained in the urine represents incompletely oxidized protein it is necessary to bring the energy balance to a computed state of nitrogen equilibrium in order to arrive at a true value for the metabolizable energy. This has been done in Table XV. The method of computation followed is the same as in previous publications from this institute.

TABLE XV.—Energy of urine, and of protein gained or lost, corrected to nitrogen equilibrium

Experiment, animal, and period Nos.	Energy of urine uncorrected for gain or loss of nitrogen *	Gain of N by body	Correc-tion N×7.45	Corrected energy	Energy of protein uncor-rected	Correc-tion, pro-tein ×5.7	Corrected energy of protein
	Cals.	Grams	Cals.	Cals.	Cals.	Cals.	Cals.
221D-885-I-----	6,595.0	-7.5	-55.9	6,539.1	256.5	-55.9	200.6
II-----	9,660.1	+4.7	+35.0	9,695.1	160.7	-35.0	125.7
III-----	6,508.7	+2.7	+20.1	6,528.8	92.3	-20.1	72.2
221D-886-I-----	6,681.8	+6.0	+44.7	6,696.5	205.2	-44.7	160.5
II-----	10,569.7	+4.2	+31.3	10,601.0	143.6	-31.3	112.3
III-----	6,517.1	+5.6	+41.7	6,558.8	191.5	-41.7	149.8
221E-885-I-----	8,045.0	+17.3	+128.9	8,173.9	591.7	-128.9	462.8
II-----	5,991.8	+3.3	+24.6	6,016.4	112.9	-24.6	88.3
221F-874-I-----	10,636.7	+2.8	+20.9	10,657.6	95.8	-20.9	74.9
II-----	7,265.3	+1.0	+7.5	7,272.8	34.2	-7.5	26.7
221F-887-I-----	9,180.7	+4.3	+32.0	9,212.7	147.1	-32.0	115.1
II-----	6,236.7	-4.9	-36.5	6,200.2	167.6	-36.5	131.1

* Corrected for loss on drying.

If there is a gain of protein by the body, then nitrogen is stored which would have appeared in the urine had actual nitrogen equilibrium been attained. In this case the correction for nitrogen is added to the energy of the urine. In case there is a loss of body protein there appears in the urine nitrogen derived from the body tissue and not from the feed. Therefore the correction is subtracted.

Having thus brought the body to nitrogen equilibrium by correcting the energy of the urine, and therefore the metabolizable energy, it becomes necessary to apply a corresponding correction to the computed energy equivalent of the protein stored or lost. This has been done in the last three columns of Table XV. It will be noted that the energy correction for the protein is always subtracted, because in computing the energy equivalent of the protein the full heat value (heat of combustion) of the protein is used, while in reality a portion of the protein energy is not utilizable, but appears in the urine in the form of incompletely oxidized nitrogenous compounds. In the case of a loss of protein, the energy equivalent is abstracted from the body and does not enter into consideration of the feed.

THE BALANCE OF ENERGY

From the data recorded on the preceding pages the balance of energy has been computed and the results set forth in Table XVI.

TABLE XVI.—The balance of energy per head and day

	221D			221D		
	885-I	885-II	885-III	886-I	886-II	886-III
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
Income.....	16,843.9	25,057.3	17,808.9	17,503.5	27,867.4	17,492.7
Outgo ^a	7,855.9	11,583.2	8,172.6	8,242.5	12,735.0	8,127.9
Metabolizable.....	8,988.0	13,474.1	9,636.3	9,261.0	15,132.4	9,364.8
Heat.....	8,380.3	9,607.4	8,816.7	8,058.6	10,474.5	7,914.1
Gain by body:						
Protein ^a	-200.6	+125.7	+72.2	+160.5	+112.3	+149.8
Fat.....	+987.1	+4,249.4	+274.6	+692.6	+4,245.6	+704.0
Error.....	-178.8	-508.4	+472.8	+349.3	+300.0	+596.9

	221E		221F		221F	
	885-I	885-II	874-I	874-II	887-I	887-II
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
Income.....	21,514.9	15,727.3	27,577.9	18,460.5	24,953.3	16,705.5
Outgo ^a	9,860.7	7,462.2	12,819.0	8,863.2	11,317.0	7,695.5
Metabolizable.....	11,654.2	8,265.1	14,758.9	9,597.3	13,636.3	9,010.0
Heat.....	9,721.7	8,735.5	10,964.2	9,076.8	10,327.2	8,395.0
Gain by body:						
Protein ^a	+462.8	+88.3	+74.9	+26.7	+115.1	-131.1
Fat.....	+2,034.0	-267.9	+3,404.8	+125.4	+2,871.9	+407.6
Error.....	-564.3	-290.8	+315.0	+368.4	+322.1	+338.5

^a Corrected for nitrogen gained or lost by the body.

Considering now cow 885, of whose erratic behavior more will be said in subsequent pages, there was a net gain of energy by the body in each of the periods of experiment 221D. Since Periods I and II were designed to approximate maintenance the gain was slight, and

in Period I a loss of protein occurred. The ration fed in Period III was a duplication of that fed in Period I. In each there was a small gain of energy, differently distributed, however, between protein and fat. There would seem to be two plausible explanations for this difference—the influence of previous feeding (a supermaintenance ration) on Period III, or the refusal of considerable quantities of feed in Period I. The writers are inclined to give the greater weight to the latter possibility.

In the lower half of this table appears an item “error.” In effect, this represents the difference between the computed and the observed heat measurements. If this error is assigned to the metabolizable energy it represents but a small percentage of the total. If it were included in the heat production its effect would be slightly increased but would still be relatively small. On the other hand, if it were considered as a part of the energy equivalent of the body gain it becomes a major factor materially affecting subsequent computations.

It is probable, however, that this item of error is a composite of a number of components of various origins. However, taking into account the painstaking attention to detail and the minute checking of the individual measurements in connection with the operation of the respiration calorimeter, and, on the other hand, the assumption that the dry matter gained or lost by the body consists of protein and fat alone, and the influence of the loss on air drying the feces on the value determined for the metabolizable energy, it would seem that the observed heat emission must be the most accurate. Therefore it is considered as the reference base in the subsequent treatment of the experimental data.

In the case of cow 886 there were also two periods (I and II) on a maintenance ration, and one period (III) on supermaintenance. In general, the results are similar to those obtained with cow 885. There is a better agreement between Periods I and III, but the “error” is considerably greater in the latter period. Judging from the appearance of the two animals, and comparing their individual reaction to the imposed experimental conditions, we should expect cow 886 to make the more efficient use of her feed. That this was actually the case is shown by a comparison of the experimental data for the two animals.

In 1921, cow 885, which had been bred immediately following her last experimental period in 1920, apparently was with calf and was so reported by the barn attendants. However, prior to the beginning of the next experimental period, she was found to be farrow. While her weight had increased since the preceding year, Table II shows the gain to have been small in relation to the feed received. Yet she appeared to be fat. She was extremely restless. Her neck became noticeably thick, as sometimes occurs in cows which have become barren. Examination by a veterinarian failed to reveal definite functional disorder. It was therefore decided to carry her along in experiment 221E. The results obtained with this cow in the maintenance period (II) are markedly unlike those obtained in the corresponding period of experiment 221D. No definite reason is apparent other than error due to the refusal of feed during all experimental periods, which, of course, renders doubtful the significance of the data obtained with the respiration calorimeter. This

cow was slaughtered after the close of experiment 221E, and, although the quantity of fat on the internal organs was unusually great, she appeared to be sound. The data obtained with this cow have been carried throughout the computations, but on account of the question as to their significance have not been included in averages.

The data presented for the two cows in experiment 221F require no special discussion. They are apparently normal in every respect.

It will be noted that the gains of energy by the body in all the maintenance periods are small, and it is assumed that they will not adversely affect the computations of net-energy values for maintenance.

In two maintenance periods, 221D-885-I and 221F-887-II, there was a slight loss of body protein. In the case of cow 885 there was a slight gain of protein in Period III, which was designed to duplicate Period I.

THE OBSERVED AND COMPUTED HEAT PRODUCTION

In Table XVII data are collected for comparison of the observed and computed heat production as obtained in these experiments. Excluding the data for cow 885, which are questionable, the agreement is reasonably good. The effect of the refused feed in the case of cow 885 is clearly apparent in the data presented in this table, and, as will be shown in the computation of the net-energy values, the heat increment per kilogram of the feed is much too low. As is shown in an analysis of the subject by Max Kriss (6) the possible sources of error in the observed as compared with the computed heat production are as indicated in the discussion following Table XVII.

TABLE XVII.—Observed and computed daily heat production

Experiment, animal, and period Nos.	Observed	Computed	Error	$\frac{\text{Computed}}{\text{Observed}} \times 100$
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals</i>	<i>Per cent</i>
221D-885-I-----	8,380.3	8,201.5	-178.8	97.87
II-----	9,607.4	9,099.0	-508.4	94.71
III-----	8,816.7	9,289.5	+472.8	105.36
221D-886-I-----	8,058.6	8,407.9	+349.3	104.33
II-----	10,474.5	10,774.5	+300.0	102.86
III-----	7,914.1	8,511.0	+596.9	107.54
221E-885-I-----	9,721.7	9,157.4	-564.3	94.20
II-----	8,735.5	8,444.7	-290.8	96.67
221F-874-I-----	10,964.2	11,279.2	+315.0	102.87
II-----	9,076.8	9,445.2	+368.4	104.06
221F-887-I-----	10,327.2	10,649.3	+322.1	103.12
II-----	8,395.0	8,733.5	+338.5	104.03

The factors entering into the observed heat production, and therefore effecting the net-energy values, are (1) the heat emission by radiation and conduction; (2) the heat emission as latent heat of water vapor; (3) the relative time spent in the standing position as compared with that spent lying; (4) the refusal of feed; (5) the gain or loss of matter by the body; and (6) the thermal environment.

In connection with the computed heat production there enter into the writers' computations the heat of combustion of the feed, of the excreta, of the methane, and of the shed hair and scurf, together

with the necessary allowances of thermal energy for fat and protein gained or lost by the body.

Considering these factors seriatim, the heat emission by radiation and conduction, constituting about 75 per cent of the total heat emission, is accurately determined; the estimation of the heat emission as latent heat of water vapor somewhat less so. Check tests by Armsby and Fries (1, p. 217-222) place the possible error of this latter determination at 6 per cent. This may be taken as the extreme, since the quantity of water vapor liberated in the check tests with alcohol is so much smaller than that measured in experiments with cows. The influence on the observed heat production of standing as compared with lying is an important factor. Animals vary greatly in the relative time spent standing and lying, and, on account of the greater energy cost of maintaining the animal in the standing position, it is necessary, in determining net-energy values of feeds or net-energy requirements of animals, to eliminate the influence of this variable. This point is treated in the discussion following Table XIX.

The influence of refusal of feed on the observed heat production is shown by the data for cow 885. The refusal of feed was about at a maximum just prior to the measurement of the heat emission in the respiration calorimeter. Hence the effect is much magnified.

The gain or loss of matter by the body influences the heat emission, in that heat is required to raise any gain from the temperature of the chamber to the temperature of the body; and, conversely, in the case of loss of body substance, heat is liberated through the cooling of the lost materials from the temperature of the body to that of the chamber.

The thermal environment would of course have a very definite influence upon the heat emission, but so long as it is maintained above the critical temperature for the subject of the investigation no error can be introduced from this source. In practical husbandry, the keeping of animals below the critical temperature constitutes a special case to which the net-energy values as determined here do not apply. It must be kept in mind, however, that the point at which physical regulation of body temperature gives way to chemical regulation is not fixed and unvarying, but is affected by the amount of the feed eaten. This becomes very important in connection with measurements of heat production on submaintenance rations and in experiments involving actual fasting.

In the case of the computed heat production some of the factors enumerated as affecting the observed heat production, notably the relative time spent standing as compared with lying, the refusal of feed, and the thermal environment, also apply. There are, however, other factors which affect only the computed heat production. These relate principally to the accuracy with which the heat of combustion of the feed and of the excreta is determined. The methane produced also becomes a material factor, as do the protein and fat gained or lost by the body. These latter considerations depend of course on the accurate determination of carbon and nitrogen; and the writers in their efforts to account for the difference between the observed and computed heat production found that very small variations in the carbon estimations are so multiplied

in the course of the computations as to materially affect the energy equivalent to the fat gained, and through this the net-energy values determined by the indirect method.

Careful consideration of the items enumerated and comparison of the available data leads to the conclusion that the observed heat production as determined here is the more accurate, but that the computed heat production may be used in the determination of net-energy values without significant compromise.

In Table XVIII the data relative to the metabolizable energy have been collected for ready reference in connection with the subsequent computation of the net energy value of the ration and the maintenance requirements of the cows.

TABLE XVIII.—*Metabolizable energy per kilogram of dry matter of rations*

Experiment, animal, and period Nos.	Dry matter eaten	Total metabolizable energy	Metabolizable energy per kilogram dry matter
	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-885-I.....	3.6674	8,988.0	2,450.8
II.....	5.4676	13,474.1	2,464.4
III.....	3.8505	9,636.3	2,502.6
Total and average.....	12.9855	32,098.4	2,471.9
221D-886-I.....	3.8131	9,261.0	2,428.7
II.....	6.0809	15,132.5	2,488.5
III.....	3.7821	9,364.8	2,476.1
Total and average.....	13.6761	33,758.2	2,468.4
221E-885-I.....	4.7012	11,654.2	2,479.0
II.....	3.4475	8,265.1	2,397.4
Total and average.....	8.1487	19,919.3	2,444.5
221F-874-I.....	5.9803	14,758.9	2,467.9
II.....	4.0032	9,597.3	2,397.4
Total and average.....	9.9835	24,356.2	2,439.6
221F-887-I.....	5.4183	13,636.3	2,516.7
II.....	3.6274	9,010.0	2,483.9
Total and average.....	9.0457	22,646.3	2,503.5

THE NET-ENERGY VALUE OF THE RATION

Only a part of the metabolizable energy of a ration is utilizable by the body. That part which is utilized can be determined, however, and it is a true measure of the actual value of the feed to the animal, from the energy standpoint. To this available portion of the metabolizable energy the term net energy is applied.

In the following computations, which are based upon the heat production of the subject of the experiment, the heat production as measured has been computed to the standard day of 12 hours standing and 12 hours lying, using the method outlined by Fries and Kriss (5) in their recent discussion of the influence of standing and lying on the metabolism of cattle. The results of this computation are presented in Table XIX.

TABLE XIX.—The observed heat production corrected to a standard day of 12 hours standing and 12 hours lying

Experiment, animal, and period Nos.	Total heat production	Time standing		Live weight	Correction of heat production for standing		Heat production corrected to 12 hours standing
		Total per day	Difference from 12 hours		Per hour	Total	
	<i>Cals.</i>	<i>Hours</i>	<i>Hours</i>	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-885-I	8,380.3	17.7	-5.7	415.2	27.4	-156.2	8,224.1
II	9,607.4	16.1	-4.1	429.7	27.4	-112.3	9,495.1
III	8,816.7	17.8	-5.8	426.4	27.4	-158.9	8,657.8
221D-886-I	8,058.6	11.0	+1.0	399.8	26.3	+26.3	8,084.9
II	10,474.5	9.2	+2.8	424.4	27.4	+76.7	10,551.2
III	7,914.1	7.9	+4.1	420.2	27.4	+112.3	8,026.4
221E-885-I	9,721.7	19.4	-7.4	443.1	28.5	-210.9	9,510.8
II	8,735.5	18.3	-6.3	434.4	27.4	-172.6	8,562.9
221F-874-I	10,964.2	11.9	+0.1	428.8	27.4	+2.7	10,966.9
II	9,076.8	11.4	+0.6	415.6	27.4	+16.4	9,093.2
221F-887-I	10,327.2	17.3	-5.3	335.2	22.9	-121.4	10,205.8
II	8,395.0	17.3	-5.3	320.5	22.9	-121.4	8,273.6

THE INCREMENT OF HEAT PRODUCTION DUE TO FEED CONSUMPTION

In the method evolved by Armsby the determination of the net energy of the feed calls for the measurement of the increase in heat production resulting from the addition of a known quantity of feed to a basal ration. To this increase in heat production the term "heat increment" has been applied. The data expressing the heat increments in this study are recorded in Table XX.

TABLE XX.—The increment of heat production due to feed consumption, computed to the standard day

Experiment, animal, and period Nos.	Dry matter eaten	Heat production, observed	Experiment, animal, and period Nos.	Dry matter eaten	Heat production, observed
	<i>Kg.</i>	<i>Cals.</i>		<i>Kg.</i>	<i>Cals.</i>
221D-885-II	5.4676	9,495.1	Difference	2.2988	2,524.8
I	3.6674	8,224.1	Difference per kg. dry matter		1,098.3
Difference	1.8002	1,271.0	Average for cow 886		1,092.9
Difference per kg. dry matter		706.0			
221D-885-II	5.4676	9,495.1	221E-885-I	4.7012	9,510.8
III	3.8505	8,657.8	II	3.4475	8,562.9
Difference	1.6171	837.3	Difference	1,2537	947.9
Difference per kg. dry matter		517.8	Difference per kg. dry matter		756.1
Average for cow 885		611.9			
221D-886-II	6.0809	10,551.2	221F-874-I	5.9803	10,966.9
I	3.8131	8,084.9	II	4.0032	9,093.2
Difference	2.2678	2,466.3	Difference	1.9771	1,873.7
Difference per kg. dry matter		1,087.5	Difference per kg. dry matter		947.7
221D-886-II	6.0809	10,551.2	221F-887-I	5.4183	10,205.8
III	3.7821	8,026.4	II	3.6274	8,273.6
			Difference	1.7909	1,932.2
			Difference per kg. dry matter		1,078.9

The data obtained in the experimental work with cow 885 have been included in Table XX, notwithstanding their obvious unreliability. It is plainly evident that the heat production of this cow is not truly related to the feed of the period, and has been rendered valueless for present purposes by the refusal of feed while on experiment. As specific evidence on this point, attention is directed to experiment 221D-885-II-III. The computed heat production in the maintenance period (III), Table XVII, is greater than in the supermaintenance period (II), an absurd finding under the conditions of feed intake and environmental temperature of the experiment.

Referring to Table XX, omitting the data obtained with cow 885, the average heat increment per kilogram of dry matter of the ration is 1,053.1 Calories.

THE COMPUTED FASTING KATABOLISM AS A MEASURE OF THE MAINTENANCE REQUIREMENT

It must be kept clearly in mind that the term fasting katabolism is not synonymous with basal katabolism, which latter involves the element of complete muscular repose. The term "fasting katabolism," as used in this paper, represents the minimum energy requirement incident to the normal daily activity of the animal, and might more accurately be termed the "24-hour normal fasting katabolism." It will also be noted that in computing the maintenance requirement to a 12-hour-standing basis, this view has been modified to some extent, for it is impossible to compare the maintenance requirements of two animals one of which, for instance, normally stands three-fourths of the time and the other one-half of the time without neutralizing the effect of this variant.

The importance of the 24-hour normal fasting katabolism in relation to feeding practice is obvious. While it is not as nearly constant as the theoretical true basal katabolism, its use is rendered imperative in experiments involving animals the muscular movements of which can not be perfectly controlled.

The foregoing data provide a basis for computing the maintenance requirement of the individual animal. The relatively constant total katabolism of a fasting animal (above the point of critical thermal environment) is a measure of the energy required for vital activities, and is consequently the minimum amount required for maintenance. Since it has been considered impracticable heretofore to measure the normal fasting katabolism of cattle directly by complete fasting experiments, the result sought has been attained indirectly from experiments in which the subjects received certain amounts of feed, as expressed in Table XXI.

TABLE XXI.—*The net energy required for maintenance per head, computed to the standard day*

Experiment, animal, and period Nos.	Dry matter eaten	Based on observed heat production *			
		Heat increment per kilo-gram dry matter	Total heat increment	Heat production	Net energy for maintenance
	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-885-I.....	3. 6674	611. 9	2, 244. 1	8, 224. 1	5, 980. 0
II.....	5. 4676		3, 345. 6	9, 495. 1	6, 149. 5
III.....	3. 8505		2, 356. 1	8, 657. 8	6, 301. 7
Average.....					6, 143. 7
221D-886-I.....	3. 8131	1, 092. 9	4, 167. 3	8, 084. 9	3, 917. 6
II.....	6. 0809		6, 645. 8	10, 551. 2	3, 905. 4
III.....	3. 7821		4, 133. 5	8, 026. 4	3, 892. 9
Average.....					3, 905. 3
221E-885-I.....	4. 7012	756. 1	3, 554. 6	9, 510. 8	
II.....	3. 4475		2, 606. 7	8, 562. 9	
Average.....					5, 956. 2
221F-874-I.....	5. 9803	947. 7	5, 667. 5	10, 966. 9	
II.....	4. 0032		3, 793. 8	9, 093. 2	
Average.....					5, 299. 4
221F-887-I.....	5. 4183	1, 078. 9	5, 845. 8	10, 205. 8	
II.....	3. 6274		3, 913. 6	8, 273. 6	
Average.....					4, 360. 0

* Heat production corrected to standard day of 12 hours standing and 12 hours lying.

It will be noted that the writers' method of computation of the fasting katabolism is as given by Armsby (3, *p. 282, sect. 374, par. 2*). This method should find general use, because it eliminates from consideration the gain or loss of energy by the body and does not make use of the metabolizable energy directly. It further serves to make clear the relationship existing between the net-energy value of the feed, the fasting katabolism, and the maintenance requirement.

The fasting katabolism has been determined for these three animals through a comparison of the effect produced by two different quantities of the same feed. The essential data involved are included in Table XXI.

The values given in Table XXI, represent the maintenance requirements of these cows for a standard day of 12 hours standing and 12 hours lying. The data are not strictly comparable, however, because the differences in live weight have not been taken into account. For comparative purposes, therefore, the values given in Table XXI for the maintenance requirement of the experimental subjects have been computed to uniform live weights of 500 kg. and 1,000 pounds, these data comprising Table XXII. The data for cow 885 have not been so computed because of their doubtful accuracy.

TABLE XXII.—*The net energy required for maintenance computed to the standard day and to uniform live weight*

Experiment, animal, and period Nos.	Live weight	Net energy for maintenance		
		As determined, observed	Per 500 kilograms live weight ^a observed	Per 1,000 pounds live weight ^a observed
	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-886-I.....	399.8	3,917.6	4,547.8	4,267.0
II.....	424.4	3,905.4	4,357.0	4,088.0
III.....	420.2	3,892.9	4,370.9	4,101.1
Average.....	414.8	3,905.3	4,425.2	4,152.0
221F-874-I.....	422.2	5,299.4	5,932.1	5,565.9
II.....				
221F-887-I.....	327.9	4,360.0	5,776.7	5,420.0
II.....				

^a Computed in proportion to the two-thirds power of the live weight.

That there should be material variation in the maintenance requirements of these three animals is to be expected from their behavior under the experimental conditions. Cow 886 was extremely quiet, was not restless when the attendants were working around her, and spent more than half of the experimental time in the lying position. Cow 874 also spent about half her time lying quietly, but when standing was much more restless. Cow 887 stood the greater part of the time, and while in the respiration calorimeter was addicted to rubbing vigorously against the side of the stall, and while lying would shift suddenly from one side to the other without arising.

In interpreting the figures given as representing the maintenance requirements of the subjects of this investigation, it must be kept in mind that direct comparison of the individuals, one with the other, is possible only after the data have been reduced to a common basis as regards standing and lying, and to a uniform live weight. This having been done, then the variations observed are due to individual differences in the animals studied.

THE NET ENERGY REQUIRED FOR MAINTENANCE COMPUTED TO THE STANDARD DAY AND TO UNIFORM LIVE WEIGHT

The average net energy required for maintenance of each of the three cows, as directly observed, and expressed in therms per 500 kg. live weight (Table XXII) is: Cow 886, 4.424 therms; cow 874, 5.932 therms; cow 887, 5.777 therms. The values as determined are not complicated by the requirements of fetal growth, since cow 886 was but two months pregnant at the beginning of the last experimental period of which she was the subject, and cows 874 and 887 had not been bred.

In experiment 221F there were two periods only with each cow. On this account the values given for the maintenance requirement of cows 874 and 887 must be taken as representative of the average of the live weights of the two periods. The difference in live weight is small in each case and has no material influence on the determined values.

THE NET-ENERGY VALUE OF THE RATION

In computing the net-energy value of the ration the writers have followed the improved procedure of Kriss (7). Table XXIII contains the data necessary to arrive at the total net energy of the ration. The items for energy gained are obtained by subtracting the heat production (computed to the standard day) from the metabolizable energy of the feed.

TABLE XXIII.—The gains of energy by the animals and the total energy of the ration

Experiment, animal, and period Nos.	Metabo- lizable energy	Based on observed heat production *			
		Observed heat production	Energy gained	Net energy for main- tenance	Total net energy of ration
	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-886-I-----	9,261.0	8,084.9	+1,176.1	3,905.3	5,081.4
II-----	15,132.4	10,551.2	+4,581.2	3,905.3	8,486.5
III-----	9,364.8	8,026.4	+1,338.4	3,905.3	5,243.7
221F-874-I-----	14,758.9	10,966.9	+3,792.0	5,299.4	9,091.4
II-----	9,597.3	9,093.2	+504.1	5,299.4	5,803.5
221F-887-I-----	13,636.3	10,205.8	+3,430.5	4,360.0	7,790.5
II-----	9,010.0	8,273.6	+736.4	4,360.0	5,096.4

* Heat production computed to standard day of 12 hours standing and 12 hours lying.

Tables XXIV and XXV present the net-energy value of the ration, as determined with three dry cows, expressed in terms of percentage utilization of the metabolizable energy, and as per kilogram dry matter of the ration. Cow 885 has been excluded from consideration on account of the persistent refusal of feed, which has been shown to vitiate the values found.

TABLE XXIV.—The percentage utilization of the metabolizable energy of the ration

Experiment, animal, and period Nos.	Metaboliz- able energy	Based on the observed heat production *	
		Total net energy of ration	Percentage utilization of the metaboliz- able energy
	<i>Cals.</i>	<i>Cals.</i>	<i>Per cent</i>
221D-886-I-----	9,261.0	5,081.4	54.9
II-----	15,132.4	8,486.5	56.1
III-----	9,364.8	5,243.7	56.0
221F-874-I-----	14,758.9	9,091.4	61.6
II-----	9,597.3	5,803.5	60.5
221F-887-I-----	13,636.3	7,790.5	57.0
II-----	9,010.0	5,096.4	56.6

* Heat production computed to standard day of 12 hours standing and 12 hours lying.

TABLE XXV.—The net energy per kilogram of dry matter of the ration

Experiment, animal, and period Nos.	Dry matter eaten	Based on the observed heat production ^a	
		Total net energy of ration	Net energy per kilo-gram dry matter
	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>
221D-886-I.....	3. 8131	5, 081. 4	1, 332. 6
II.....	6. 0809	8, 486. 5	1, 395. 6
III.....	3. 7821	5, 243. 7	1, 386. 5
221F-874-I.....	5. 9803	9, 091. 4	1, 520. 2
II.....	4. 0032	5, 803. 5	1, 449. 7
221F-887-I.....	5. 4183	7, 790. 5	1, 437. 8
II.....	3. 6274	5, 096. 4	1, 405. 0

^a Heat production computed to standard day of 12 hours standing and 12 hours lying.

The percentages of utilization of metabolizable energy given in Table XXIV show good agreement. The maximum is 61.6 per cent, the minimum 54.9 per cent, and the average for the series 57.7 per cent.

It would appear from these data that individual differences exist in the expense of utilization of the metabolizable energy. These differences represent unlike energy expenditure, on various accounts, in the utilization of the feed, and also any such error as there may be in the determination of the maintenance requirement, the metabolizable energy, and the heat increment.

The experimental animals were apparently normal, average individuals, and the data for percentage utilization seem to satisfactorily represent the net energy of the ration as a proportion of that which is metabolizable.

The data presented in Table XXV represent the net-energy value of the ration expressed as Calories per kilogram of dry matter of the feed. The average value found is 1,418.2 Calories.

In determining the rations to be fed during this series of experiments, the net-energy value of the ration was calculated from the average values for the hay and grain components as given by Armsby.

(2) These data follow:

	Therms per kg. dry matter.
Oats.....	1. 637
Corn meal.....	2. 113
Wheat bran.....	1. 297
Linseed meal.....	2. 152
Mixed grain.....	1. 730
Alfalfa hay.....	0. 824
In ration used.....	1. 364

The value obtained in experiment 221F-874-I (Table XXV) seems to be high, but no valid reason can be found for excluding it. Comparing the average value obtained in this series of experiments with that computed from Armsby's average values, the former is 4 per cent higher.

SUMMARY

The net energy required for maintenance by three dry cows was determined in a series of respiration calorimeter experiments to be 4.150, 5.420, and 5.566 therms, respectively, per 1,000 pounds of live weight. The lowest value (4.150 therms) is ascribed to the individuality of the subject, which was a cow of unusually quiet disposition. The other two cows appeared to be in no way unusual, and their maintenance requirements are not abnormally low, though appreciably less than Armsby's published average of 6 therms. It would seem, therefore, that this standard figure is sufficiently high.

Since two of the three determinations of maintenance requirement fall within the range of variation of the previous figures for steers published from the institute, there is in these results no definite warrant for anticipating the establishment of a maintenance requirement for cows differing from that of steers.

The applicability of these results to feeding under conditions of practice is indicated by the fact that the average maintenance requirement of 6 therms of energy for steers, as determined at the institute, has been found to be a satisfactory measure of the maintenance requirement, under conditions of practice, not only of steers but also of cows.

In these experiments there were gains of energy by all three subjects on rations computed to supply the maintenance requirements in accord with the 6-therm average.

The net-energy value of a ration composed of 40 per cent alfalfa hay and 60 per cent grain mixture, the latter consisting of 30 parts wheat bran, 30 parts ground oats, 30 parts corn meal, and 10 parts old process linseed meal, was found to be 1.418 therms per kilogram of dry matter of the ration, as determined by direct measurement of the heat production of the animals.

A method of approximating the apparent digestibility of a ration where it is impracticable to collect the dung and urine separately is reported.

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REVISED NET-ENERGY VALUES OF FEEDING STUFFS FOR CATTLE¹

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INTRODUCTION

One of the chief purposes of the work with the respiration calorimeter at the Pennsylvania Institute of Animal Nutrition has been to determine the total expenditure of energy by animals in the utilization of feed for maintenance and production, the difference between the gross energy and the energy losses and expenditures of utilization constituting the net-energy value.

The methods employed and the results obtained with steers, during the years 1902 to 1915, have been reported in articles by Armsby and Fries (1, 2, 3, 4, 6, 7, 8, 9).² The derivation of these values involves comparisons of heat production in periods of unlike feed consumption, these comparisons being dependent upon uniform conditions as to expenditure of energy for maintenance, especially as determined by the time spent by the animal in the standing and the lying positions.

In recent studies at this institute (10, 11), the evidence obtained has disclosed imperfections in the treatment of the experimental data and in the computations which have led to the published net-energy values. These studies have led (1) to a new method of correcting the heat production of the experimental animal to a standard day, as to standing and lying, and (2) to an important change in the method of computation of the net-energy values. These improved procedures, which have been described in detail elsewhere (10, 11), are of such importance as to necessitate the recalculation of all of the net-energy determinations of feeds for steers which have been published from this institute, and the corrected results are set forth in this paper.

EXPERIMENTS AND ANIMALS

The experiments involved in this recomputation include 12 series, comprising 71 experimental periods, with 9 different steers, varying in age from 20 months to approximately 60 months at the beginning of the several experiments.

This covers all of the net-energy values of feeds for steers which have been determined at this institute (1, 2, 3, 4, 6, 7, 8, 9), except the results of two series of experiments (Nos. 190 and 208) with young, growing steers, which have been omitted from this paper for reasons which will be explained.

Data relating to the steers which served as experimental subjects in these investigations comprise Table I.

¹ Received for publication Feb. 18, 1925; issued January, 1926.

² Reference is made by number (italic) to "Literature cited" p. 1098.

TABLE I.—Descriptive data relating to the experimental subjects

Animal	Breed	Number of experiments in which used	Age at beginning of experiment	Average live weight
			<i>Months</i>	<i>Kg.</i>
I.....	Grade Shorthorn.....	174	36	408
		179	48	528
		186	60	572
A.....	Aberdeen Angus.....	200	23	408
		207	35	510
B.....	Scrub.....	200	25	303
		207	37	380
D.....	Grade Hereford.....	210	21	331
		211	33	449
F.....do.....	209	21	300
G.....	Hereford.....	211	28	379
H.....	Shorthorn.....	212	20	345
		216	22	383
J.....do.....	217	33	512
		217	37	649
K.....do.....	220	24	501

NEW METHOD OF CORRECTING THE HEAT PRODUCTION TO A STANDARD DAY OF 12 HOURS STANDING AND 12 HOURS LYING

The question of the influence of position on the metabolism of cattle has been studied and discussed in many publications by Armsby and Fries (3, 4, 5, 6), who devised and used a method of correcting the heat production to a standard day, as to standing and lying, based on the rate of heat elimination as directly measured in these different positions. A recent critical study of this problem by Fries and Kriss (10) has revealed this method to be seriously in error, first, because of the inaccurate division of the heat between the intervals of standing and lying, due to several factors, but principally to the large capacity of the apparatus to store heat while the animal is lying and to radiate this heat during the subsequent period of standing, and, second, because this method, in effect, involves the assumption that the energy required for maintenance and for feed utilization contribute either at uniform or at characteristic rates to the total heat production of standing and of lying, and are equally affected by lapse of time spent in these positions.

This study has led not only to the discovery and estimation of the instrumental errors, but also to a change of conception of the causes for increased heat production while standing as compared with lying, and to the formulation of a new and simple method of correcting the daily heat production, as measured, to a standard day as to standing and lying.

That an animal should produce more heat while standing than while lying, other conditions remaining equal, is readily understandable on the basis of greater muscular activity in the standing position. This excess of heat, which represents a part of the maintenance requirement of net energy, was found with a fasting dry cow weighing 400 kg. to be 26.3 Cals. per hour.

With this figure as a basis we correct the heat production, as measured, to represent a standard day of 12 hours standing and 12 hours lying, thus establishing a basis for determining the difference

in heat production between periods compared, in relation to the differences in feed. This factor (26.3 Cals. per hour), representing an animal of 400 kg. live weight, is modified for animals of other weights in accord with the two-thirds power of the live weight, as follows:

Live weight	Factor	Live weight	Factor	Live weight	Factor	Live weight	Factor
<i>Kg.</i>	<i>Cals.</i>	<i>Kg.</i>	<i>Cals.</i>	<i>Kg.</i>	<i>Cals.</i>	<i>Kg.</i>	<i>Cals.</i>
300-----	21.7	375-----	25.2	450-----	28.5	525-----	31.5
325-----	22.9	400-----	26.3	475-----	29.5	550-----	32.5
350-----	24.1	425-----	27.4	500-----	30.5	575-----	33.5

The correction to the standard day of 12 hours standing and 12 hours lying is computed by determining the difference in hours between 12 (the standard) and the time actually spent in the standing position, and multiplying this difference by the factor corresponding to the live weight of the animal. This correction is subtracted from the heat production as measured, or is added thereto, according to whether the animal stood more or less than 12 hours during the calorimeter day. For example, the daily heat production of a steer weighing 501 kg. was 10,754 Cals. The steer spent 9.25 hours per day in the standing position. The correction for standing is $(12 - 9.25) \times 30.5 = +84$ Cals., and the heat production corrected to the standard day of 12 hours standing and 12 hours lying is $10,754 + 84 = 10,838$ Cals. If this animal had stood 14.75 hours per day the correction would be $(12 - 14.75) \times 30.5 = -84$ Cals., and to correct the daily heat production to the standard day of 12 hours standing and 12 hours lying the 84 Cals. would be subtracted. It should be noted that for the determination of the heat increment caused by the feed, and, consequently, for the determination of the net-energy value of the feed, the length of the standard day is immaterial. For a detailed discussion of this method and the derivation of the factors, the reader is referred to the original publication (10).

DATA FOR THE DETERMINATION OF NET-ENERGY VALUES

In order to compute the net-energy value of a feeding stuff, it is necessary to have from each of two or more periods of differing intake of the feed of interest, (1) the daily dry matter of the feed eaten, (2) the metabolizable energy of the ration (energy of the feed minus the energy of the excreta), and (3) the daily heat production by the animal, corrected to the standard day of 12 hours standing and 12 hours lying.

In recomputing the net-energy values of the feeds for which such values have been published from this institute, the data for dry matter of feed, for metabolizable energy, and for the daily heat production, as measured, remain as previously reported, except where arithmetical or other errors of work have been discovered;³ but inasmuch as the new method of correcting the heat production to a

³ Attention is called to an error in the value for the average metabolizable energy per kilogram of dry matter of timothy hay in experiment 174 as reported in the following: H. P. Armsby and J. A. Fries, Net energy values of feeding stuffs for cattle, Jour. Agr. Research 3: 441, 444, 1915. The value reported there is 1,674 Cals. The correct value is 1,953 Cals. For the linseed meal in this experiment the writers used the values for metabolizable energy, heat increment, and net energy given in the following: H. P. Armsby, The nutrition of farm animals, 743 p., illus., New York, 1917.

standard day differs from that previously used, the data for the "corrected" heat production differ, of course, from those which have been published. In order to make it possible to follow the recomputation of the net-energy values the complete data are presented in Table II. The gains of energy given in the last column of the table are computed by subtracting the corrected heat production from the metabolizable energy.

TABLE II.—Data for computation of net-energy values

Experiment No. and feeding stuffs	Animal	Period	Time spent standing per day	Dry matter eaten		Metabolizable energy of ration	Heat production corrected to 12 hours standing	Gain of energy by animal
				Roughage	Concentrates			
			<i>Hours</i>	<i>Kg.</i>	<i>Kg.</i>	<i>Cals.</i>	<i>Cals.</i>	<i>Cals.</i>
174. Timothy hay and linseed meal.....	I	A	13. 68	2. 8795	0. 3578	6, 651	9, 151	-2, 500
Do.....	I	B	16. 35	4. 0180	. 3547	9, 491	10, 161	-670
Do.....	I	C	13. 63	5. 1243	. 3561	11, 198	10, 705	+493
Do.....	I	D	14. 47	6. 0858	. 3544	12, 106	11, 405	+701
179. Red clover hay.....	I	1	14. 73	4. 4590	-----	8, 449	11, 420	-2, 971
Do.....	I	2	15. 12	3. 1439	-----	6, 154	10, 003	-3, 849
Red clover hay and corn mea.....	I	3	13. 52	3. 1628	. 7347	8, 238	10, 468	-2, 230
Do.....	I	4	18. 36	3. 1864	3. 4508	17, 973	14, 413	+3, 560
186. Red clover hay.....	I	1a	20. 80	2. 9333	-----	5, 922	10, 597	-4, 675
Do.....	I	1b	24. 00	2. 9333	-----	5, 922	11, 321	-5, 399
Do.....	I	2a	16. 28	5. 0253	-----	10, 690	11, 268	-578
Do.....	I	2b	17. 60	5. 0253	-----	10, 690	11, 113	-423
Do.....	I	3a	14. 87	4. 1391	-----	8, 614	10, 605	-1, 990
Do.....	I	3b	17. 38	4. 1391	-----	8, 614	10, 677	-2, 062
200. Timothy hay and grain mixture								
No. 1.....	A	1	12. 58	2. 6079	1. 7920	10, 721	9, 324	+1, 397
Do.....	A	2	13. 87	2. 6309	4. 1727	17, 290	12, 823	+4, 467
Timothy hay.....	A	3	13. 71	2. 6472	-----	4, 939	7, 407	-2, 468
Do.....	A	4	12. 20	4. 4245	-----	7, 981	8, 204	-223
Timothy hay and grain mixture								
No. 1.....	B	1	11. 35	2. 4331	1. 1947	8, 502	8, 951	-449
Do.....	B	2	10. 86	2. 4539	2. 1290	10, 922	9, 743	+1, 179
Timothy hay.....	B	3	13. 62	2. 4703	-----	4, 751	6, 893	-2, 142
Do.....	B	4	7. 93	3. 8051	-----	7, 100	7, 468	-368
207. Timothy hay and grain mixture								
No. 1.....	A	1	12. 09	2. 9349	1. 9962	12, 061	10, 171	+1, 890
Do.....	A	2	15. 44	2. 9487	4. 7590	20, 553	14, 035	+6, 518
Timothy hay.....	A	3	11. 78	2. 9742	-----	6, 235	7, 780	-1, 545
Do.....	A	4	11. 75	4. 8920	-----	10, 157	9, 501	+656
Timothy hay and grain mixture								
No. 1.....	B	1	11. 50	2. 7608	1. 3978	9, 443	9, 527	-84
Do.....	B	2	10. 98	2. 7743	2. 6769	14, 021	11, 532	+2, 489
Timothy hay.....	B	3	10. 31	2. 7983	-----	5, 687	7, 925	-2, 238
Do.....	B	4	9. 44	4. 6299	-----	9, 446	9, 478	-32
209. Alfalfa hay and grain mixture No. 2.....	F	1	6. 4	1. 4615	3. 0406	11, 362	9, 096	+2, 266
Do.....	F	2	8. 0	. 8935	1. 8620	7, 331	7, 476	-145
Do.....	F	3	7. 7	. 5361	1. 1120	4, 438	6, 430	-1, 992
Alfalfa hay.....	F	4	8. 2	6. 1737	-----	11, 399	11, 081	+318
Do.....	F	5	7. 8	3. 5622	-----	6, 623	7, 763	-1, 140
Do.....	F	6	11. 1	2. 2264	-----	3, 838	6, 737	-2, 899
210. Corn stover.....	D	1	12. 70	4. 3353	-----	8, 523	9, 457	-934
Do.....	D	2	9. 02	3. 5475	-----	6, 986	8, 263	-1, 277
Do.....	D	3	8. 58	2. 5628	-----	4, 923	7, 193	-2, 270
211. Mixed hay.....	D	1	7. 12	6. 2042	-----	11, 348	11, 710	-362
Mixed hay and hominy chop.....	D	2	9. 62	1. 7473	1. 7637	9, 435	9, 665	-230
Do.....	D	3	5. 85	3. 9105	3. 9488	21, 406	14, 123	+7, 283
Mixed hay.....	D	4	8. 70	3. 4983	-----	6, 958	9, 302	-2, 344
Do.....	D	5	9. 66	1. 7864	-----	3, 276	8, 020	-4, 744
Do.....	G	1	9. 52	6. 0920	-----	12, 054	11, 761	+293
Mixed hay and corn meal.....	G	2	10. 00	. 7903	1. 5419	6, 887	8, 245	-1, 358
Do.....	G	3	6. 72	2. 3832	4. 6437	18, 894	13, 444	+5, 450
Mixed hay.....	G	4	8. 34	3. 1485	-----	6, 217	9, 029	-2, 812
Do.....	G	5	7. 27	1. 6078	-----	3, 018	6, 983	-3, 965
212. Alfalfa hay.....	H	1	10. 13	6. 6383	-----	13, 337	11, 134	+2, 203
Alfalfa meal.....	H	2	9. 50	6. 6707	-----	13, 238	10, 946	+2, 292
Alfalfa hay.....	H	3	7. 05	5. 3203	-----	10, 650	9, 843	+807
Alfalfa meal.....	H	4	9. 14	5. 4078	-----	10, 661	9, 767	+894
Alfalfa hay.....	H	5	8. 26	3. 0524	-----	6, 510	7, 394	-884
Alfalfa meal.....	H	6	8. 98	3. 1549	-----	6, 587	6, 868	-281

TABLE II.—Data for computation of net-energy values—Continued

Experiment No. and feeding stuffs	Animal	Period	Time spent standing per day	Dry matter eaten		Metabolizable energy of ration	Heat production corrected to 12 hours standing	Gain of energy by animal
				Roughage	Concentrates			
			Hours	Kg.	Kg.	Cals.	Cals.	Cals.
216. Alfalfa hay and starch-----	J	1	8. 79	6. 2486	2. 5724	20, 860	15, 623	+5, 237
Do-----	J	2	9. 26	2. 6454	1. 1050	8, 854	9, 600	-746
Do-----	J	3	7. 57	3. 7477	1. 5511	12, 019	10, 924	+1, 095
Do-----	J	4	9. 51	1. 7540	. 7260	5, 628	8, 026	-2, 398
Alfalfa hay-----	J	5	8. 97	7. 8930	-----	15, 100	12, 818	+2, 282
Do-----	J	6	7. 13	6. 1278	-----	11, 954	10, 954	+1, 000
Do-----	J	7	8. 49	3. 5016	-----	6, 902	8, 315	-1, 413
217. Alfalfa hay and grain mixture No. 3.	J	1	10. 15	1. 5076	2. 9553	12, 269	10, 954	+1, 315
Do-----	J	2	13. 37	3. 0884	6. 0579	25, 398	16, 604	+8, 794
Do-----	^a J	3	17. 56	3. 4395	6. 7872	28, 747	21, 002	+7, 745
Do-----	^a J	4	13. 01	1. 7543	3. 4613	14, 338	14, 187	+151
220. Red clover hay-----	K	1	11. 12	5. 9523	-----	11, 365	12, 151	-786
Do-----	K	2	11. 62	3. 9415	-----	7, 972	10, 300	-2, 328
Red clover hay and corn meal-----	K	3	9. 25	1. 3279	2. 6017	11, 292	10, 838	+454
Do-----	K	4	10. 27	2. 2717	4. 3630	18, 132	13, 899	+4, 233
Do-----	K	5	13. 81	. 9087	1. 7475	7, 383	10, 001	-2, 618

^a Fattened.

REVISED METHOD OF COMPUTATION OF NET-ENERGY VALUES

A detailed description of the new method of computing net-energy values, supplemented by a number of examples, showing the advantage of this method over those previously used, has already been published by one of the writers (11), and to avoid unnecessary repetition the description here will be general.

The distinguishing feature of this method is that it makes possible the computation of a net-energy value of a feed for each of a series of experimental periods, instead of giving only one value representing results of two or more periods, as accomplished by the earlier methods. The computation is carried out according to the following general procedure:

- (1) The daily gain of energy by the animal in each experimental period of a series is computed by subtracting the daily heat production ⁴ from the metabolizable energy of the ration (see Table 2, last column).
- (2) The heat increment per kilogram of dry matter of a feed is computed by comparing the dry matter and the heat production of each period with the dry matter and the heat production of the other periods. The difference in heat production divided by the difference in dry matter of feed consumed (kilogram) gives the increment of heat production per kilogram of dry matter. The values thus obtained are averaged.
- (3) The total heat increment caused by the feed is computed by multiplying the total dry matter of the feed (kilogram) by the average heat increment per kilogram of dry matter, as obtained in (2).
- (4) The net energy required for maintenance is computed by subtracting the total heat increment caused by the ration, in each period,

⁴ Corrected to 12 hours standing and 12 hours lying. This is also implied in the subsequent uses of this term.

from the heat production of the corresponding period. The average of the several determinations is considered to represent the maintenance requirement of the animal during the series of experimental periods.

(5) The total net energy of each ration is computed by adding to the gain, as obtained in (1), the average net energy required for maintenance, as obtained in (4).

(6) The net-energy value per kilogram of dry matter of feed is computed by dividing the total net energy of the feed by the dry matter (kilogram) of the feed.

(7) The percentage utilization of the metabolizable energy is computed by dividing the total net energy of the feed by its total metabolizable energy, and multiplying the result by 100.

INCREMENTS OF HEAT PRODUCTION PER KILOGRAM OF DRY MATTER OF FEED

The feeds used in the experiments under consideration consisted in some cases of coarse fodder alone, and in others of a mixture of roughage and concentrates (Table II). The increments of heat production per kilogram of roughage alone, or of a uniform mixture of hay and grain, are computed directly, as outlined above, by comparing the periods in which different quantities of the same feed, or of the same mixture of feeds, were consumed.

In the experiments following No. 207 the rations consisted of a uniform mixture of feeds, in the different periods. The increments of heat production, therefore, are computed per kilogram of the mixture, these values being directly usable for the computation of the maintenance requirement of the animal.

In the earlier experiments, however, up to and including No. 207, the hay and grain were not fed in the same proportion in the different periods. In these the heat increments per kilogram of the grain have been computed by making use of the hay periods in the comparisons.

The heat increment values of the feeds, as obtained by the use of the heat production corrected to 12 hours standing and 12 hours lying, according to the new method, and the averages of each series, are set forth in Table III.

TABLE III.—Increments of heat production per kilogram of dry matter of feed

[Values in parenthesis are not included in averages]

Experiment No.	Animal	Feeding stuff	Periods compared	Heat increment per kilogram of dry matter
				<i>Cals.</i>
174.....	I	Timothy hay.....	D and A.....	704
		Do.....	C and A.....	693
		Do.....	B and A.....	889
		Do.....	D and B.....	602
		Do.....	C and B.....	491
		Do.....	D and C.....	730
		Average.....		685
179.....	I	Red clover hay.....	1 and 2.....	1, 077
186.....	I	Corn meal.....	2 and 4.....	^a 1, 264
		Red clover hay.....	1a and 3a.....	(7)
		Do.....	2a and 3a.....	(748)
		Do.....	1a and 2a.....	(321)
		Do.....	1b and 3b.....	(-534)
		Do.....	2b and 3b.....	(492)
200.....	A	Do.....	1b and 2b.....	(-99)
		Timothy hay.....	3 and 4.....	(448)
		Grain mixture No. 1.....	1 and 3.....	(1, 080)
	B	Do.....	2 and 1.....	(1, 466)
		Do.....	2 and 3.....	(1, 300)
		Timothy hay.....	3 and 4.....	(431)
207.....	A	Grain mixture No. 1.....	1 and 3.....	(1, 736)
		Do.....	2 and 1.....	(838)
		Do.....	2 and 3.....	(1, 342)
	B	Timothy hay.....	3 and 4.....	897
		Grain mixture No. 1.....	1 and 3.....	^a 1, 215
		Do.....	2 and 1.....	1, 394
209.....	A	Do.....	2 and 3.....	^b 1, 319
		Average.....		1, 309
		Timothy hay.....	3 and 4.....	848
	B	Grain mixture No. 1.....	1 and 3.....	^a 1, 169
		Do.....	2 and 1.....	1, 559
		Do.....	2 and 3.....	^b 1, 355
		Average.....		1, 361
210.....	F	Alfalfa hay.....	4 and 5.....	1, 271
		Do.....	5 and 6.....	768
		Do.....	4 and 6.....	1, 100
	F	Average.....		1, 046
		Alfalfa hay and grain mixture No. 2.....	1 and 2.....	927
		Do.....	2 and 3.....	945
211.....	D	Do.....	1 and 3.....	934
		Average.....		935
		Corn stover.....	1 and 2.....	1, 516
	D	Do.....	2 and 3.....	1, 087
		Do.....	1 and 3.....	1, 277
		Average.....		1, 293
211.....	D	Mixed hay.....	1 and 4.....	890
		Do.....	4 and 5.....	750
		Do.....	1 and 5.....	835
	D	Average.....		825
		Mixed hay and hominy chop.....	2 and 3.....	1, 025
		G	Mixed hay.....	1 and 4.....
G	Do.....	4 and 5.....	1, 328	
	Do.....	1 and 5.....	1, 066	
	Average.....		1, 107	
G	Mixed hay and corn meal.....	2 and 3.....	1, 107	

^a The same value will be obtained by comparing periods 1 and 4.
^b The same value will be obtained by comparing periods 2 and 4.

TABLE III.—Increments of heat production per kilogram of dry matter of feed—Continued

Experiment No.	Animal	Feeding stuff	Periods compared	Heat increment per kilogram of dry matter
212	H	Alfalfa hay	1 and 3	<i>Cals.</i> 980
		Do	3 and 5	1,080
		Do	1 and 5	1,043
		Average		1,034
		Alfalfa meal	2 and 4	934
		Do	4 and 6	1,287
		Do	2 and 6	1,160
		Average		1,127
		Alfalfa hay	5 and 6	1,056
		Do	6 and 7	1,005
216	J	Do	5 and 7	1,025
		Average		1,029
		Alfalfa hay and starch	1 and 2	1,188
		Do	1 and 3	1,334
		Do	1 and 4	1,198
		Do	3 and 2	855
		Do	2 and 4	1,239
		Do	3 and 4	1,028
		Average		1,140
		Alfalfa hay and grain mixture No. 3	1 and 2	1,206
217	J	Do	3 and 4	1,360
220	K	Red clover hay	1 and 2	921
		Red clover hay and corn meal	3 and 4	1,132
	K	Do	4 and 5	980
		Do	3 and 5	(658)
		Average		1,056

• Fattened.

VARIABILITY OF HEAT-INCREMENT VALUES

The variations in the heat increments per kilogram of dry matter exhibited in Table III call for a consideration of their true significance and of several factors possibly contributing to apparent discrepancies.

These variations are not to be considered in the same light as variations in other determinations, for example, of digestibility or of metabolizable energy. We may have, for instance, in two periods, independent determinations of metabolizable energy per kilogram of feed, but, in connection with the same, only one heat-increment value. On the other hand, three periods on the same feed make possible three comparisons, and, therefore, as many determinations of heat increment; and four periods make possible six comparisons, and, therefore, six determinations of heat increment, while no one of these heat-increment values represents the result of a single period. Variations in heat increments, therefore, can not be referred to the heat production of individual periods. The extent of these variations, and their effects on the average heat increment per kilogram of feed, depend not only upon the magnitude of the errors in the values rep-

resenting heat production, but also upon the periods in which the errors occurred, and upon the differences in feed between the periods compared. This may be illustrated by the following hypothetical example involving three experimental periods:

Let H_1 , H_2 , and H_3 represent the heat production of Periods I, II, and III, respectively. The ration of Period III is the smallest. The difference in dry matter consumed between Periods II and III is 1 kg.; between I and II, 3 kg.; and between I and III, 4 kg. The heat increments per kilogram of dry matter would therefore be as follows:

Periods compared	Heat increment per kilogram dry matter
I and II.....	$\frac{H_1 - H_2}{3}$
II and III.....	$\frac{H_2 - H_3}{1}$
I and III.....	$\frac{H_1 - H_3}{4}$

Let three cases be considered:

- (1) If H_1 alone is in error by ± 300 Cals.
- (2) If H_2 alone is in error by ± 300 Cals.
- (3) If H_3 alone is in error by ± 300 Cals.

If h represents the true heat increment per kilogram of dry matter of the feed, we will have the following heat-increment values in the three cases just mentioned:

Periods compared	Difference in feed (dry matter)	Heat increments per kilogram of dry matter		
		Case 1	Case 2	Case 3
I and II.....	<i>Kg.</i> 3	<i>Cals.</i> $h \pm 100$	<i>Cals.</i> $h \mp 100$	<i>Cals.</i> h
II and III.....	1	h	$h \pm 300$	$h \mp 300$
I and III.....	4	$h \pm 75$	h	$h \mp 75$
Average.....		$h \pm 58$	$h \pm 67$	$h \mp 125$

The difference between the extreme heat-increment values in case 1 is 100 Cals., while it is 400 Cals. in case 2, and 300 Cals. in case 3, although the magnitude of the assumed error in the heat production is the same in each case. The effect of this assumed error on the average heat-increment value is also different in the three cases.

Especial attention is called to the relative magnitude of the deviations from the true value (h) in the different comparisons of the periods in which the error is involved. The deviation is greatest where the difference in feed is smallest. On this account it seems very desirable that the feed of the different periods to be compared shall differ in quantity as widely as practicable; and in some cases in which the evidence is clear as to the location of the errors it seems permissible to exclude from the average the heat-increment values based upon small differences in feed.

Accordingly, we have excluded Period III of experiment 179 from the computation of the average heat-increment value of corn meal, because of the small quantity of meal represented in this period, and we have also excluded from the average the heat-increment value of clover hay and corn meal as obtained by comparing Periods III and V in experiment 220, because of the small difference in feed.

Among the sources of error in the heat production, and consequently in the heat increments, in some of the experiments here considered, two require especial mention. These are (1) the refusal of feed in some of the periods of heavy consumption, and (2) the probability that the temperature of the calorimeter was below the critical temperature for the animal in some of the periods in which the subjects on submaintenance rations suffered extensive losses of energy from the body.

The amounts of feed refused have been reported in the publication of the details of these experiments (1, 2, 3, 4, 6, 7, 8, 9) and will not be repeated here. In experiments 209 and 210, which have been published only in condensed form (6) and not in full detail, there were considerable amounts of feed refused during some of the periods. During Period IV, experiment 209, the animal was offered daily 6.8037 kg. of dry matter of alfalfa hay, but the average daily amount eaten was only 6.1737 kg. (See Table II.) During Period I of the same experiment the animal rejected 147.6 grams of dry matter of hay and 9.3 grams of grain. In experiment 210 the animal was offered daily during Period I 7.6303 kg. of dry matter of corn stover, and during Period II 4.2040 kg., but the average daily amounts eaten during these periods were 4.3353 kg. and 3.5475 kg., respectively.

In comparing the heat production and the rations of the different periods, for the derivation of the heat increments, the amounts of feed refused were in each case subtracted from the total offered. It is, however, readily understandable that such a procedure may not always be effective in accomplishing the desired correction for refusal of feed, when it is considered that the balance of matter represents an average of 10 days' feeding, while the heat production is derived from only two days. It is clear that the success or failure of such an attempt at correction of the heat production must depend to a large extent on whether the refusal of feed was uniform throughout the entire digestion period, including the calorimeter days.

In connection with the effect of refusal of feed on the heat production, its effect on the determination of metabolizable energy, which also is involved in the computation of net-energy values, must also be mentioned.

A refusal of feed occurring early enough in the collection period to be wholly reflected in the fecal outgo of this period would be corrected for by subtraction of the amount of the refused feed from the amount of the feed offered, but if the decrease in fecal outgo consequent upon the refusal of feed does not all occur prior to the termination of the collection period, the attempt at correction of the balance of matter by subtraction of the amount of feed refused from the amount of feed offered would at best be incomplete, and, under some conditions, might actually introduce into the balance a new error of the extent of the entire amount of the refused feed thus subtracted from the amount of feed offered. In the experimental practice of this institute the

result has been, in all probability, rather commonly at least, intermediate between these extremes, there being no way to determine positively the net effect of this effort to correct for refusal of feed. In fact, refusal of feed introduces most perplexing complications, and, if considerable in amount, is fatal to the significance of the results.

For present purposes the writers leave this matter as it stands, with a citation of the published figures representing amounts of feed refused, and an acknowledgment of the fact that this has been a source of error—commonly a minor one, it is true, but sometimes extensive.

As for the bearing of the critical temperature in this connection, there is evidence to suggest, in some periods of restricted feed intake, that the heat incident to the vital processes and the utilization of the feed was insufficient to maintain the normal temperature of the animal, the subject being obliged, therefore, to oxidize body substance to make good the deficit. In such a case the heat increment would exceed the amount derived from the feed, and so would be in error as related to the feed alone.

In the present lack of definite evidence as to what the critical temperature for cattle is, under the various conditions in accord with which it is believed to vary, we are unable to say positively that this factor is responsible for certain anomalous results obtained, but the presumptive evidence is of such weight, in the writers' judgment, as to warrant withholding some of these results until further information as to critical temperatures shall remove the question as to their significance.

In other experiments, however, in which there was ground for suspecting error on this account, it was possible to use the results by computing the maintenance requirements from the results of other experiments with the same subjects, as will be explained in the following paragraph.

MAINTENANCE REQUIREMENT OF NET ENERGY

In computing the net energy required for maintenance, the general procedure as outlined was followed, except in two series of experiments, namely Nos. 186 and 200. In these cases, on account of the ground for suspicion that the temperature in the calorimeter was below the critical for the subjects in some of the periods, we have computed the maintenance requirements from data obtained in other experiments with the same animals. In so doing the writers computed this quota in proportion to the two-thirds power of the live weight. Thus the maintenance requirement of steer I in experiment 186 was computed from the maintenance of this animal as determined in experiment 179, while the maintenance requirements of steers A and B in experiment 200 were computed from the maintenance requirements of these animals as determined in experiment 207.

In this connection it should be stated that the reason for not including in this paper the experiments with the young growing steers, namely experiments Nos. 190 and 208, to which reference has already been made, is that these experiments are also subject to the same suspicion of having been affected by subcritical temperatures, and, as the writers have no adequate basis for computing the net-energy

requirement for maintenance of these young animals, the net-energy estimations derivable from the results are withheld for the time being.

Table 4 gives the maintenance requirements of net energy, as determined in the different periods, and the averages for the series. It should be noted that in averaging the maintenance requirements for the series of experimental periods only one value is given for two periods in those cases in which only two periods were the basis for determining the heat-increment value of the feed. This is done because in such cases only one comparison is possible, and only one heat-increment value, therefore, is obtained. The value for maintenance must be considered to be the same in the two periods.

TABLE IV.—Maintenance requirements of net energy

Ex- peri- ment No.	Ani- mal	Net energy required for maintenance							
		In successive periods							Average
		Cals.	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.	
174	I	6, 794	7, 027	6, 812	6, 855				6, 872
179	I	6, 617			6, 619				6, 618
186	I								^a 6, 981
200	A								^b 4, 365
200	B								^b 4, 692
207	A	4, 925	5, 160	5, 113					5, 066
207	B	5, 284	5, 536	5, 552					5, 457
209	F	4, 887	4, 900	4, 889	4, 623	4, 037	4, 408		4, 624
210	D	3, 851	3, 676	3, 879					3, 802
211	D	6, 592	6, 067		6, 416	6, 546			6, 405
211	G	5, 017	5, 664		5, 544	5, 203			5, 357
212	H	4, 270	3, 428	4, 342	3, 672	4, 238	3, 312		3, 877
216	J	5, 567	5, 325	4, 883	5, 199	4, 696	4, 648	4, 712	5, 004
217	J	5, 573							5, 573
217	*J			7, 094					7, 094
220	K	6, 669		6, 688	6, 893	7, 196			6, 861

* Computed from experiment 179. ^b Computed from experiment 207. ^c Fattened.

VARIATIONS IN THE VALUES FOR MAINTENANCE

An examination of the values for maintenance requirement of net energy in each of the series of experiments from which the averages are derived, shows that the differences are apparently due (Table IV) not to differences in kind of ration used (hay, or hay and grain), in the different periods of the same series, or to variations in live weight of the animal, but to accumulated errors of work. Thus, in experiment 211 the maintenance requirement of net energy for steer D seems to be less in Periods II and III, on hay and grain rations, than in the other periods in which hay alone was fed, while the maintenance requirement of steer G appears to be greater in the hay and grain periods than in the hay periods. In experiments 209, 216, and 220, the maintenance requirements computed from the hay and grain rations are, in general, greater than those computed from the hay rations, but in some of these the differences are only slight. In experiment 212 the maintenance requirements computed from the alfalfa meal rations (Periods II, IV, VI) are considerably less than those computed from the alfalfa hay rations (Periods I, III, V). These differences can hardly be due to difference in kind of feed. As to the possible effect of variations in the live weight of the animals, in the different periods, on the results of the computation

of the maintenance requirement of net energy, the writers would suggest that, with the exception of experiment 217, the experimental periods were short (three or four weeks only), while the changes in the amount and kind of feed consumed were considerable. It seems probable, therefore, that the larger part of the variation in weight must be ascribed to differences in the content of the alimentary tract, and that the actual effect on the basal metabolism was but slight. In view of all these considerations, the writers are inclined to ascribe the apparent variations in the maintenance requirement of net energy, in the different experimental periods of a series, in Table IV, to the cumulative effect of experimental errors, and to consider the average of the several determinations as applying to each of the individual experimental periods.

In experiment 217 the animal was actually fattened during the transition period of more than three months, between Periods II and III, and made considerable gain in weight (see Table I). In this experiment the maintenance requirements of the animal in the unfattened condition and the fattened condition have been computed separately, the values being as shown for Periods II-III and III-IV, respectively.

NET-ENERGY VALUES OF FEEDS

The total net energy of the rations is obtained, by addition, of the average of the determinations of net-energy requirement for maintenance, as reported in Table IV, and the gains of energy, as given in Table II. These total net-energy values, divided by the dry matter of the rations, give the net-energy values per kilogram of dry matter of the rations. The net-energy values of the grains are computed by subtracting from the total net energy of the mixed ration the net-energy equivalent of the hay, by the use of a value previously determined in the hay rations. The remainder represents the net energy of the grain, and by dividing this by the kilograms of dry matter of grain the net-energy value per kilogram of grain is obtained.

We have also computed, in each case, the percentage utilization of the metabolizable energy. This is another way of expressing the net-energy value of a feed, and is obtained by dividing the net energy of the feed by its metabolizable energy (instead of the dry matter), and multiplying the result by 100. The net-energy values, on both bases, are presented in Table V.

TABLE V.—*Net-energy values of feeding stuffs*

[Values in parentheses are not included in averages]

Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy	Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
Timothy hay:		<i>Cals.</i>	<i>Per cent</i>	Corn stover:		<i>Cals.</i>	<i>Per cent</i>
174.....	I-A.....	1,275	65.98	210.....	D-I.....	662	33.65
	I-B.....	1,371	65.45		D-II.....	712	36.14
	I-C.....	1,301	65.90		D-III.....	598	31.14
	I-D.....	1,130	62.36	Average.....		657	33.64
200.....	A-III.....	(717)	(38.41)				
	A-IV.....	(936)	(51.90)	Grain mixture No. 3:			
	B-III.....	(1,032)	(53.67)	217.....	J-I.....	1,833	58.02
	B-IV.....	(1,136)	(60.90)		J-II.....	1,874	58.55
207.....	A-III.....	1,184	56.47	Average.....		1,854	58.29
	A-IV.....	1,170	56.34				
	B-III.....	1,150	56.60	Grain mixture No. 1:			
	B-IV.....	1,172	57.43	200.....	A-I.....	(1,853)	(55.95)
Average, omitting experiment 200.....		1,219	60.82		A-II.....	(1,526)	(51.11)
Alfalfa hay:				200.....	B-I.....	(1,344)	(41.27)
209.....	F-IV.....	800	43.35		B-II.....	(1,508)	(51.20)
	F-V.....	978	52.60	207.....	A-I.....	1,754	59.00
	F-VI.....	775	44.95		A-II.....	1,705	56.34
212.....	H-I.....	916	45.59	207.....	B-I.....	1,551	56.72
	H-III.....	881	43.99		B-II.....	1,765	56.43
	H-V.....	981	45.99	Average, omitting experiment 200.....		1,694	57.12
Alfalfa meal:							
212.....	H-II.....	925	46.61	Grain mixture No. 2:			
	H-IV.....	882	44.76	209.....	F-I.....	1,857	64.77
	H-VI.....	(1,140)	(54.61)		F-II.....	1,997	65.09
Alfalfa hay:					F-III.....	1,957	62.75
216.....	J-V.....	923	48.25	Average.....		1,937	64.20
	J-VI.....	980	50.23				
	J-VII.....	1,026	52.03	Red clover hay and corn meal:			
Average, including alfalfa meal.....		915	47.12	220.....	K-III.....	1,862	64.78
Corn meal:					K-IV.....	1,672	61.18
179.....	I-III.....	(2,314)	(79.22)		K-V.....	1,597	57.47
	I-IV.....	2,165	63.11	Average.....		1,710	61.14
211.....	G-II.....	2,149	61.93				
	G-III.....	1,882	61.29	Hominy chop:			
220.....	K-III.....	2,257	67.65	211.....	D-II.....	2,490	71.48
	K-IV.....	1,977	63.13		D-III.....	2,455	69.06
	K-V.....	1,863	58.18	Average.....		2,473	70.27
Average.....		2,049	62.55				
Red clover hay:				Starch:			
179.....	I-I.....	818	43.16	216.....	J-I.....	1,610	47.58
	I-II.....	881	45.00		J-II.....	1,517	45.19
186.....	I-Ia.....	(786)	(38.94)		J-III.....	1,574	51.61
	I-Ib.....	(540)	(26.73)		J-IV.....	(1,231)	(40.34)
	I-IIa.....	(1,274)	(59.90)	Average.....		1,567	48.13
	I-IIb.....	(1,305)	(61.35)				
	I-IIIa.....	(1,206)	(57.93)	Mixed hay and corn meal:			
	I-IIIb.....	(1,188)	(57.10)	211.....	G-II.....	1,715	58.07
220.....	K-I.....	1,021	53.45		G-III.....	1,538	57.20
	K-II.....	1,150	56.86	Average.....		1,627	57.64
Average, omitting experiment 186.....		968	49.62				
Mixed hay:				Alfalfa hay and grain mixture No. 3:			
211.....	D-I.....	974	53.25	217.....	J-I.....	1,543	56.14
	D-IV.....	1,160	58.34		J-II.....	1,571	56.57
	D-V.....	930	50.70		*J-III.....	1,451	51.62
	G-I.....	927	46.86		*J-IV.....	1,389	50.53
	G-IV.....	808	40.94	Average.....		1,489	53.72
	G-V.....	866	46.12				
Average.....		944	49.37				

*Fattened.

TABLE V.—*Net-energy values of feeding stuffs*—Continued

Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy	Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
Red clover hay and corn meal: 220-----	K-II-----	<i>Cals.</i> 1, 862	<i>Per cent</i> 64. 78	Alfalfa hay and grain mixture No. 3: 217-----	J-I-----	<i>Cals.</i> 1, 543	<i>Per cent</i> 56. 14
	K-IV-----	1, 672	61. 18		J-II-----	1, 571	56. 57
	K-V-----	1, 597	57. 47		^a J-III-----	1, 451	51. 62
Average-----		1, 710	61. 14		^a J-IV-----	1, 389	50. 53
Mixed hay and hominy chop: 211-----	D-II-----	1, 759	65. 45	Alfalfa hay and starch: 216-----	J-I-----	1, 161	49. 09
	D-III-----	1, 742	63. 94		J-II-----	1, 135	48. 09
Average-----		1, 751	64. 70		J-III-----	1, 151	50. 74
Alfalfa hay and grain mixture No. 2: 209-----	F-I-----	1, 530	60. 64		J-IV-----	1, 051	46. 30
	F-II-----	1, 625	61. 10	Average-----		1, 125	48. 56
	F-III-----	1, 597	59. 31				
Average-----		1, 584	60. 35				

AVERAGE NET-ENERGY VALUES

It will be noted in Table V that some of the values are in parenthesis. These values are excluded from the averages. Among these are all of the net-energy values obtained for clover hay in experiment 186, and for timothy hay in experiment 200, although some of the results fall within the range of variation of the values obtained in other experiments with the same feeds. These have been excluded for the reason that they rest upon a somewhat less substantial basis than the other determinations, the maintenance requirement having been computed from results of other experiments with the same animals.

The results on corn meal in Period III of experiment 179 (2), and the results on starch in Period IV of experiment 216 (9) are given in parentheses because of the small quantities of these feeds represented.

The values for alfalfa meal in Period VI of experiment 212 have been excluded from the average because of the extent of their divergence from the other values of the same series.

The net-energy values of grain mixture No. 3 in experiment 217 have been computed from the mixed feed of hay and grain in Periods I and II, representing the unfattened condition of the animal, by the use of the net-energy value of the hay obtained in experiment 216 with the same animal.

For convenience of reference, the average net-energy values of Table V have been assembled in Table VI.

TABLE VI.—Average net-energy values of feeding stuffs

Feeding stuffs	Net energy per kilogram of dry matter	Percentage utilization of metab- olizable energy
	<i>Cals.</i>	<i>Per cent</i>
Timothy hay.....	1,219	60.82
Red clover hay.....	968	49.62
Mixed hay.....	944	49.37
Alfalfa hay ^a	915	47.12
Corn stover.....	657	33.64
Corn meal.....	2,049	62.55
Grain mixture No. 1 ^b	1,694	57.12
Grain mixture No. 2 ^c	1,937	64.20
Grain mixture No. 3 ^d	1,854	58.29
Hominy chop.....	2,473	70.27
Starch.....	1,567	48.13
Mixed hay and corn meal, 1:2.....	1,627	57.64
Red clover hay and corn meal, 1:2.....	1,710	61.14
Mixed hay and hominy chop, 1:1.....	1,751	64.70
Alfalfa hay and grain mixture No. 2, 1:2.....	1,584	60.35
Alfalfa hay and grain mixture No. 3, 1:2.....	1,489	53.72
Alfalfa hay and starch, 2:4:1.....	1,125	48.56

^a Includes alfalfa meal.^b 1 part wheat bran, 3 parts corn meal, 3 parts old process linseed oil meal.^c 6 parts corn meal, 3 parts crushed oats, 1 part coarsely ground linseed cake.^d 6 parts corn meal, 2 parts wheat bran, 1 part cottonseed meal.

SUMMARY

The latest evidence in relation to the determination of the net energy of feeds, in accord with Armsby's conception, having led (1) to a new and greatly improved method of correcting the heat production of the experimental animal to a standard day, in relation to the position of the subject as to standing and lying, and (2) to an important change in the method of computation of the net-energy value, it has become necessary to recompute and to correct the net-energy values of feeds, for the maintenance and body increase of steers, which have been published from the Institute of Animal Nutrition of the Pennsylvania State College (1, 2, 3, 4, 6, 7, 8, 9). The corrected net-energy values are as assembled in Table VI.

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STUDIES IN WESTERN YELLOW PINE NURSERY PRACTICE¹

By DONALD R. BREWSTER and J. A. LARSEN, *formerly of the Northern Rocky Mountain Forest Experiment Station, Forest Service, United States Department of Agriculture*

INTRODUCTION

In 1912 and 1913, when nursery experiments were started under direction of the then "Priest River" Forest Experiment Station, at Priest River, Idaho, and elsewhere, western yellow pine (*Pinus ponderosa*) was one of the principal species being planted on a large scale in the northern Rocky Mountain region and millions of plants were being raised each year in the Forest Service nurseries; but comparatively little careful study had been made to determine the best methods of handling this stock in the nursery to obtain good quality at a minimum cost. The experience of the years following 1909, when the nursery work was first undertaken on a large scale, demonstrated beyond question the need of experimentation in order to produce a better and more uniform quality of stock and to avoid the delays, uncertainties, and losses due to lack of exact information.

The investigative work undertaken at this time was limited to those phases of the western yellow pine nursery practice most suitable for study at a small experimental nursery with limited facilities and at a considerable distance from the large nurseries operating on a commercial scale. These phases were: (1) Depth of covering seed in the seed bed; (2) methods of sowing seed in the seed bed; (3) degree of shade to use on the seed bed; (4) amount of water to use on the seed bed, with or without cultivation.

Such phases as the proper amount of seed to sow, the best season to sow, the need for fertilizing the soil, development of a good root system, and methods and season of transplanting, could at that time be studied better at the larger nurseries, either because of the large scale on which it was necessary to conduct the investigations or because the problems were more or less local and could best be solved at the nursery where the results were to be applied.

While the four phases selected for study are to a certain extent interrelated with climatic and soil conditions, these conditions at the field station in northern Idaho sufficiently resemble those at the Savenac Nursery in northwestern Montana to permit putting into practice there the results obtained at the Idaho station. The mean monthly temperatures for the growing season at Savenac average only one or two degrees lower than those at Priest River. Percentage of sunshine and wind velocity are probably somewhat greater at Savenac, but the same general regional climate prevails at both places.

¹ Received for publication Apr. 14, 1925; issued December, 1925.

All studies were conducted at the Priest River field station, with the exception of duplicate experiments in the study of the depth of cover begun at the Savenac and Boulder nurseries in 1912 and at the Trapper Creek nursery in 1914, under the direction of the experiment station.

The 1912 depth of cover bed at Priest River was located at the Benton Flat nursery, a dry flat with a sandy loam soil and thin sandy subsoil. The 1913 studies of depth of cover, and all studies on the other three phases, were located in a group of sixteen 4 by 12 foot beds known as the Meadow nursery. These beds were on a gentle southerly slope just above a flat meadow near Benton Creek. They had about a 10 per cent gradient, and were terraced to make their surfaces level. The natural soil was a fine silt loam 12 to 18 inches deep with a reddish color and a marked tendency to become compact or caked on the surface. It was underlaid by a light gray, claylike silt subsoil so compact and hard that water penetrated very slowly. To overcome the heavy nature of the soil, sharp granitic sand was mixed with the top 6 inches of each bed, rendering the soil loose and loamy and largely overcoming the tendency to pack on the surface.

In all of the experiments included in this series, counts of germination and of loss were made approximately once a week throughout the germinating period of the first season in the seed bed. A survival count was made at the end of the season to check the totals which had been carried forward. Each seedling, as soon as its germination was noted, was marked by sticking a toothpick in the ground just north of it. Toothpicks were colored to indicate the month in which germination occurred. Dead seedlings and the corresponding toothpicks were removed at each weekly count and the cause of death noted. Damping-off was found to be the most prevalent cause of death of first-year seedlings.

In the fall or following spring, at the time of removing the seedlings from the seed beds, measurements and weights were obtained of representative plants to show relative development under different conditions. From one to five seedlings, depending on the number available, and representing as nearly as possible the average development of each lot of plants, were preserved for future record. Photographs were made of these specimen plants in the winter of 1916-17 to illustrate differences in development due to varying treatment in the seed beds.

DEPTH OF COVERING IN SEED BED

SUMMARY

Depth of covering layer has a direct effect on the temperature and moisture of the soil in contact with the seed, upon the amount of mechanical obstruction to the growth of the stem toward the surface, and on the favorable conditions for the development of injurious fungi as well as the vigor and resistance of the plants to fungus attack. It is because of these effects that variations in depth cause differences in germination and survival of seedlings.

In all of the experiments the shallower covers very uniformly show a more rapid germination, a larger total germination, a smaller loss

from damping off and other causes, and a better development of the plants at the end of the season, when compared with the deeper cover. The contrasts are so marked that the use of the shallower cover can be unhesitatingly recommended for use with western yellow pine in all nurseries in this region, at least where artificial watering is possible. The best showing was, on the whole, made by the $\frac{1}{4}$ -inch and $\frac{3}{8}$ -inch depths. A cover varying between these two, with an average of $\frac{5}{16}$ inch, should be adopted as standard and be adhered to as closely as equipment and facilities will permit, in order to secure greatest economy of seed and space in raising desirable plants.

While it may be difficult to secure and maintain an exactly uniform and minutely regulated depth of cover, owing to mechanical difficulties in applying the cover and because of the washing of surface particles in sprinkling the beds, it has been found possible, by the development (at the Savenac nursery) of a special machine for covering, and by care in watering, to keep variations usually within $\frac{1}{8}$ inch and never more than $\frac{1}{4}$ inch. This makes it feasible to regulate the depth of cover to a range which can be maintained in large scale practice and one within which best results in germination and survival may be obtained.

PROCEDURE

In the spring of 1912, western yellow pine seed, collected on the Bitterroot National Forest in 1911, was sown in the Benton Flat nursery and at the Savenac and Boulder nurseries. Depths of $\frac{1}{2}$ inch and $\frac{3}{4}$ inch were used, and seed was sown in plots 2 feet square, each containing 500 seeds. Counts were made once a week and seedlings were pulled as counted, thus restricting the record to germination alone.

In 1913 the work was carried out with greater completeness. One bed in the Meadow nursery was thoroughly spaded and worked over until in a good condition for sowing. The bed was then divided by wood strips into two series of six plots each for use with six different depths of cover. In one series the plots were 2 feet square for broadcast sowing, and in the other they were 1 by 4 feet for drill sowing. Thus by duplicating the different depths of cover with the two methods of sowing it was possible to make a combined study of depth and method of sowing in the same bed so as to bring out facts in regard to each phase as well as their relation to each other.

Fresh western yellow pine seed was used. It was collected and extracted at the experiment station the previous fall (1912). Sowing was done June 6, the comparatively late sowing being due partly to delay in getting the new nursery in shape for sowing and partly to the fact that the season of 1913 was from three to four weeks later than the average. About 375 seeds to the square foot were sown, or an amount of seed sufficient to produce an estimated stand of 200 seedlings in each drill, or 200 to the square foot, on the basis of the greenhouse figures. The seed used for each plot was accurately weighed to within a limit of error of 4 seeds or 1 per cent of the good seeds sown, the same weight being used for all plots. Seed was sown as uniformly as possible. The drills were 3 inches apart, 4 drills to a plot, lengthwise.

One plot in each of the two series was covered to one of the following depths: $\frac{1}{4}$ inch, $\frac{3}{8}$ inch, $\frac{1}{2}$ inch, $\frac{5}{8}$ inch, $\frac{3}{4}$ inch, and 1 inch. Clean sharp sand, dug from the sterile subsoil at Benton Flat, was used for covering. After the seed was sown and lightly pressed into the surface several toothpicks were inserted in each plot so that their points projected above the surface to a height exactly equal to the depth of cover desired. The sand was then put on and smoothed off level with the tops of the toothpicks. The lath partitions between plots prevented sand from washing from the plots with deep cover to those with shallower cover.

For want of definite knowledge as to shading requirements, the bed was given one-half shade from the time of sowing to the end of the season.

Water was applied to the bed by means of a hose and fine spray nozzle toward the close of each day during the dry period, except immediately after rains, in a moderate quantity sufficient to keep the upper soil layers moist enough for good growth.

Counts of germination and loss were made once a week, and a final survival count was made October 4, using the colored toothpick method.

In the spring of 1914 the seedlings were removed for transplanting and 50 representative seedlings were mechanically selected for measurement by taking every third, fourth, or fifth plant, depending on the total number in the plot. Figures were obtained on the following points: Length of root in inches, length of stem in inches, weight of 50 tops (fresh and surface dried) in grams. Typical specimens were pressed, and those in the broadcast series were photographed, in February, 1917.

The rest of the seedlings were transplanted at the Benton Flat nursery in the spring of 1914, using a standard "Yale" board. Survival records of transplants were obtained in the fall of 1915, together with measurements of the height and diameter at the ground line of every tenth plant.

One bed sown at the Trapper Creek nursery on the Bitterroot National Forest May 27, 1914, contained 4 plots, each 1 by 4 feet, each plot containing about 600 seeds. One plot was covered to each of the following depths: $\frac{1}{4}$ inch, $\frac{3}{8}$ inch, $\frac{1}{2}$ inch, and $\frac{5}{8}$ inch. Seed was sown in drills 3 inches apart, 4 drills per plot, lengthwise. Counts of germination and survival were made June 15, July 1, 15, and 22, and again October 1. Owing to the conditions at Trapper Creek, it was not possible to control this experiment as carefully or have as complete records as for the 1913 experiment at Priest River. The value of this experiment lies chiefly in confirming, in a general way, the Priest River results, and in throwing an interesting sidelight upon the effect of different climatic conditions and methods of irrigation on the optimum depth of cover for yellow pine.

DATA

The germination figures obtained in the 1913 series at the three nurseries—Priest River, Savenac, and Boulder—are summarized in Table I.

TABLE I.—Germination percentage under different depths of cover, 1912

Depth of cover	Comparison of nurseries			
	Savenac	Priest River	Boulder	Average
1/2 inch.....	Per cent 50.6	Per cent 41.4	Per cent 31.8	Per cent 41.0
3/4 inch.....	48.6	40.8	27.2	38.9

The differences in these figures for the different nurseries may be partly explained by the fact that sowing was done May 1 at Savenac, May 15 at Priest River, and June 5 at Boulder, because of differences in the beginning of the growing season and the time when it was possible to do the sowing. Climatic differences at the three places are indicated in Table II.

TABLE II.—Comparison of temperatures and precipitation at nurseries, 1912

Nursery	Monthly mean temperature		Mean maximum temperature		Precipitation	
	May	June	May	June	May	June
Priest River.....	° F. 51.7	° F. 60.5	° F. 80	° F. 97	Inches 2.68	Inches 2.14
Savenac.....	50.6	59.0	83	97	2.33	1.12
Boulder.....	46.2	57.4	75	90	4.03	1.32

The temperature figures furnish one evident reason why germination at Boulder was lowest. The higher mean maximum air temperature at Savenac was probably one of the contributing factors in producing greatest germination at that place, when combined with the advantage of early sowing.

While the differences between germination at the two depths are quite small, the fact that they are consistently in favor of the shallower depth of 1/2 inch at all three places and under different conditions is excellent evidence, when taken in connection with similar evidence from later experiments, to show that 1/2 inch is a better depth than 3/4 inch for covering yellow pine seed.

The germination figures obtained in the 1913 study at Priest River are shown graphically in Figures 1 and 2, where the effects of the different depths of cover and of the two methods of sowing are compared.

The following points in regard to germination are brought out by these curves:

(1) Greatest germination was attained with a 1/4-inch depth of cover, which was the shallowest; and the second greatest was with 3/8-inch depth. The 1/2-inch and 5/8-inch covers showed about the same amount, due to the fact that on the 5/8-inch broadcast plot the depth of cover was reduced by washing, with a consequent marked increase in germination for that plot. The deeper sowings showed still less, the 3/4-inch plot taking fifth place, and the 1-inch plot sixth place.

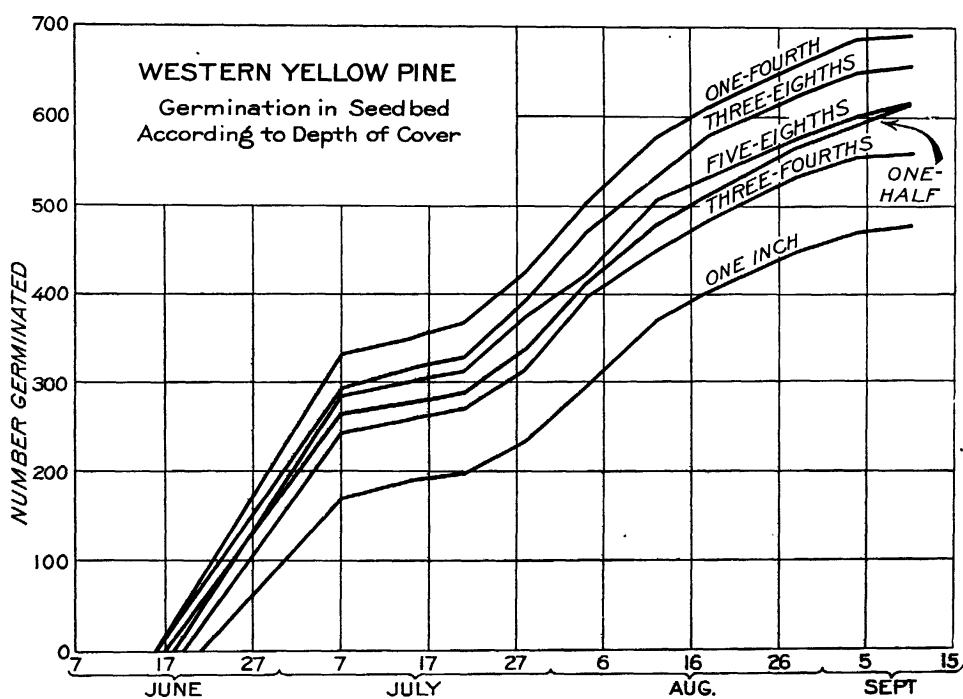


FIG. 1.—Weekly march of germination, 1913: Depth of cover

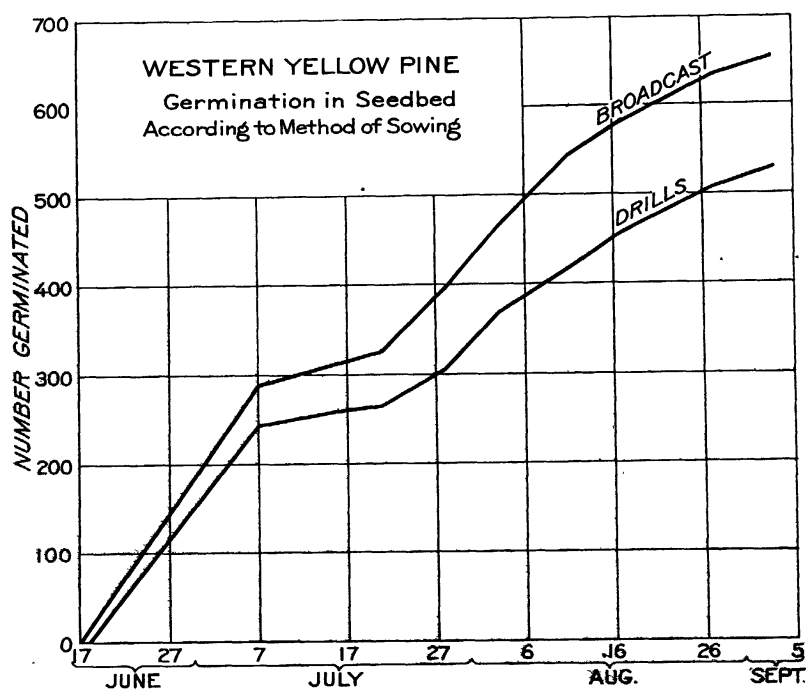


FIG. 2.—Weekly march of germination, 1913: Method of sowing

(2) Germination started five days earlier under the shallowest cover than under the deepest.

(3) The shallowest sowings, $\frac{1}{4}$ inch and $\frac{3}{8}$ inch, showed the most rapid rate of germination.

(4) The period of most active germination occurred in the last half of June and the first half of July, from three to four weeks after sowing.

The survival and development of the stock in the seed bed at the end of the season, and the results in the transplant bed, are shown in Table III.

TABLE III.—Survival and development of western yellow pine in seed bed and transplant bed, under different depths of cover and methods of sowing, 1913

Depth of cover	Method of sowing	Seed beds						Transplant beds			
		Total germination	Survival	Average measurements				Spring, 1915, survival	Fall, 1915		
				Length of root	Length of stem	Weight of root	Weight of stem		Survival	Diameter of stem	Height of stem
		Number	Per cent	Inches	Inches	Grams	Grams	Per cent	Per cent	Mm.	Mm.
¼ inch -----	Drills -----	714	76.5	9.30	2.20	3.40	5.80	79.0	76.2	4.90	6.00
	Broadcast ..	702	79.8	9.20	1.80	2.40	3.85	83.0	81.0	4.50	4.90
	Average ..	708	78.1	9.25	2.00	2.90	4.82	81.5	79.1	4.70	5.45
⅓ inch -----	Drills -----	577	79.0	9.30	2.40	3.00	5.43	83.2	82.5	4.90	5.60
	Broadcast ..	776	83.1	9.50	1.80	2.35	3.60	86.0	83.0	4.90	6.00
	Average ..	676	81.4	9.40	2.10	2.68	4.52	84.5	83.8	4.90	5.80
½ inch -----	Drills -----	656	77.3	9.70	2.40	3.08	5.65	80.0	77.7	4.80	5.90
	Broadcast ..	565	85.3	10.60	2.10	3.13	4.45	86.5	82.2	4.70	5.70
	Average ..	610	81.0	10.15	2.25	3.10	5.05	83.9	80.6	4.75	5.80
⅝ inch -----	Drills -----	505	59.8	10.10	1.90	3.15	4.20	87.0	84.0	4.90	5.90
	Broadcast ..	* 733	68.2	11.50	2.10	3.25	4.65	89.9	87.3	4.50	5.40
	Average ..	* 619	64.8	10.80	2.00	3.20	4.42	88.9	85.7	4.70	5.65
¾ inch -----	Drills -----	449	65.2	9.50	2.00	2.70	4.60	92.7	84.3	4.50	5.80
	Broadcast ..	694	63.1	11.10	2.00	3.05	4.15	56.5	55.5	4.30	5.00
	Average ..	572	64.0	10.30	2.00	2.88	4.38	64.5	61.9	4.40	5.40
1 inch -----	Drills -----	366	64.8	9.10	1.90	2.55	4.70	83.3	83.3	4.20	4.90
	Broadcast ..	621	72.3	9.70	2.00	2.50	3.90	85.6	84.6	4.90	5.10
	Average ..	494	69.4	9.40	1.95	2.52	4.30	85.1	84.5	4.55	5.00
Summary											
Total or average for ¼, ⅓, and ½ inch:											
Drills -----		1,947	77.5	9.43	2.33	3.16	5.63	80.8	78.9	4.83	5.83
Broadcast ..		2,043	82.6	9.77	1.90	2.63	3.97	84.9	82.1	4.70	5.53
Total or average for all depths:											
Drills -----		3,267	71.7	9.50	2.13	2.98	5.06	83.1	80.4	4.70	5.68
Broadcast ..		4,091	75.1	10.27	1.97	2.78	4.10	80.9	78.3	4.63	5.35

* Depth of cover decreased by washing, causing increase in germination.

The averages for the broadcast and drilled plots shown in Table III bring out the following points:

- (1) The survival in the seed bed is highest for the ⅓-inch covered plots, followed closely by the plots with ½-inch and ¼-inch cover.
- (2) The seedlings from the plots covered ⅝ inch have the longest roots, those from ¾ inch and ½ inch covers coming next.
- (3) The seedlings from the plots covered ½ inch show the largest stems and the heaviest tops.
- (4) The heaviest roots were produced on the seedlings from the plots covered ⅝ inch. The comparison between length and weight

of roots is not exact, however, since roots were simply dug in the ordinary way with a spade and it was impossible to obtain the entire root.

(5) The figures for survival in the transplant bed, both after the first year and at the end of the second year, are too irregular to indicate a clearly defined influence of the depth of cover on the survival of transplants.

(6) The transplants from the plots with shallow covers show a slightly better development than those from the more deeply covered plots. This is probably due to the earlier and more vigorous germination in the shallow plots.

The notes taken on the condition of the seedlings when removed from the seed bed are as follows:

Western yellow pine at 1/4 inch: Seedlings dark green and thrifty, but apparently many seeds failed to germinate because either entirely or partly on the surface. Radicals of some appear to rise into the air leaving crown in the soil. Practically every germination for several weeks after July 10 was lost through damping-off and drought.

- At 3/8 inch: Fine, healthy appearance.
- At 1/2 inch: Good, thrifty appearance.
- At 5/8 inch: Fairly healthy appearance. Damping-off very active.
- At 3/4 inch: Seedlings pale green and inclined to be poor form. Damping-off bad.

At 1 inch: Seedlings rather deformed, pale and unthrifty, tending to damp-off.

Since practically all loss was due to damping-off, the lower survival rate in the deeper covers seems to indicate greater susceptibility to the attacks of damping-off fungi. The root systems of the stock from the lesser depths were more bushy, although perhaps not so long, as those from the 5/8-inch and 3/4-inch plots.

Germination and survival figures for the experiment conducted at Trapper Creek nursery in 1914 are shown in Table IV.

TABLE IV.—Depth of cover germination and survival percentages, Trapper Creek, 1914

Depth of cover	Germination at successive observation dates, in percentages of total germination					Total germination of seed sown		Survival of seedlings germinated	
	June 15	July 1	July 15	July 22	Oct. 1				
	Per cent	Per cent	Per cent	Per cent	Per cent	No.	Per cent	No.	Per cent
1/4 inch.....	12.3	70.5	15.3	0.0	1.9	163	27.2	158	96.9
3/8 inch.....	2.2	72.5	24.8	0.0	0.5	177	29.5	154	87.0
1/2 inch.....	3.0	86.2	7.2	1.8	1.8	166	27.7	157	94.6
5/8 inch.....	0.0	81.7	15.6	0.7	2.0	148	24.7	135	91.2

The following points are brought out by Table IV:

- (1) Although the earliest germination occurred on the plots with shallower cover, the plots with deeper cover showed equal or greater total germination by July 1.
- (2) Greatest total germination was under the 3/8-inch cover, with the 1/2-inch and the 1/4-inch next in order, and the 5/8-inch, the deepest, in the last place.
- (3) The least depth, 1/4 inch, showed the highest percentage of plants surviving at the end of the season, with 1/2 and 5/8 inch next in order, and 3/8 inch last. In percentage of seed sown, however, survival at 3/8 inch is very much better than at 5/8 inch.

METHODS OF SOWING SEED

SUMMARY

Sowing in long open drills permits pruning of roots in place and facilitates cultivation. Because of the economy of space and time, broadcast sowing has been generally adopted at the Savenac and Boulder nurseries.

The experiments at Priest River in 1913-14 show, from the standpoint of economy of seed, that the evidence is consistently in favor of broadcast sowing. This method produced highest germination, highest survival, and best development of the plants.

Because of the lack of facilities for large-scale production, it was impossible to investigate accurately the difference in economy of time and space between broadcast and drill sowing. These questions have, however, already been worked out at the Savenac nursery, with results in favor of broadcasting for northern Rocky Mountain conditions. It may, therefore, be definitely concluded that for conditions in this region where watering is not possible, and where it is not necessary to cultivate between the rows to conserve moisture, yellow pine seed should be sown broadcast.

PROCEDURE

The principal experiment in this series was identical with that carried on at the Priest River station in 1913 in the depth-of-cover study in the Meadow nursery. It is therefore unnecessary to repeat the description of operation already given.

In order to obtain data on the effect of fall sowing upon methods, two more beds were sown in the Meadow nursery in the fall of 1913. No. 1 was sown broadcast and No. 2 in drills, using the same seed used in the spring-sowing experiment, covered with clean sand to a depth of $\frac{3}{8}$ -inch after careful spading and preparation of the beds. Protection and watering were as for spring sowing. Six representative areas, 1 foot square in the broadcast bed and one drill in the drilled bed, were selected for intensive counts which were made weekly during 1914.

DATA

The results of the 1913 spring-sown study at Priest River have already been assembled in Figures 1 and 2 and Table III. The summary of Table III has been prepared to show contrasts due to differences in method of sowing rather than depth of cover. Reference is made to these data, in regard to the essential points brought out, as follows:

(1) Germination in the broadcast plots was consistently greater than in the drilled plots, or 25 per cent more for all depths. For the three shallower depths, however, the average of the broadcast plots was only 5 per cent greater, so that for the depths which will be used in practice the advantage over the drills is very slight. The greater difference in the deeper sowings was due perhaps to the fact that the cover on the three broadcast plots was decreased somewhat by watering, thus probably increasing the germination.

(2) During the first month germination in the drilled plots was more rapid. By the middle of July the broadcast plots were in the lead in point of numbers. It evidently takes the seedlings in the

broadcast plots a little longer to break through the ground, possibly because in the drills the combined lifting force of many seedlings is concentrated along a narrow line. Figure 2 shows graphically the rate and amount of germination.

(3) Survival in the seed bed, in percentage of the number that germinated, was higher for the broadcast plots, both for the average of the three lesser depths (5.1 per cent) and also for the average of all depths (3.4 per cent). The comparatively higher survival in the broadcast plots is due to greater susceptibility of the seedlings along the drills to attack from damping-off.

(4) In general, the stock from the broadcast plots showed a longer root and a shorter top than that from the drills, and the tops and roots were, on the whole, lighter in weight. The broadcast method produced the largest seedlings, except for the $\frac{3}{8}$ -inch and $\frac{1}{4}$ -inch depths, and the drill method produced the heaviest plants, except for the $\frac{5}{8}$ -inch depth.

(5) The stock raised by the broadcast method showed a higher survival in all cases, except for the lot raised with $\frac{3}{4}$ -inch cover. The latter made such a radically different showing from any of the rest that it can safely be assumed to have been injured either during transplanting or later.

(6) Measurements of both seedlings and transplants show that the stock raised in the drills had longer roots and tops. This is due to greater competition for light and root room in the case of seedlings crowded together in drills. It really means that the drills produced an inferior grade of stock compared to the broadcast plots and is an argument in favor of broadcast sowing.

A comparison of the amount of germination in the fall-sown beds is of no value, for the reason that mice got in during the winter, in spite of protective screens, and did more or less damage. Therefore the germination figures have not been tabulated. The total amount of germination during the season was 25 per cent greater in the drills than in the broadcast beds, but this may only indicate that more of the broadcasted seed was eaten than of that in the drills.

Loss in the drilled bed was almost 50 per cent greater than in the broadcast bed, showing a marked advantage in favor of broadcasting.

Plants from the drills had longer, more spindling tops than those from the broadcast bed, an additional argument in favor of the latter method.

DEGREE OF SHADE

SUMMARY

As earlier nursery experience in other regions had shown that the tender first-year seedlings of many conifers were benefited by being partially shaded from the direct rays of the sun, it was the practice to use shade on western yellow pine beds in the earlier nursery work in this region. By 1913, however, when this study was started, shading had been discontinued for yellow pine at Savenac and Trapper Creek nurseries, for general observations indicated that it was unnecessary. The experiments at Priest River were undertaken to obtain definite information on this point.

The basis for drawing conclusions was the effect of the different degrees of shade upon germination, survival, growth, and development of seedlings, and survival and growth of transplants.

Germination is affected by the influence of shade upon temperature and moisture content of the soil. Shade influences survival by reducing excessive water loss of the young plants in transpiration, by decreasing the surface temperature and evaporation from the soil, and by making conditions more favorable for the growth and spread of damping-off fungi. Shade affects the survival and growth of transplants indirectly through its effect upon the rate of germination, since the seed which germinates earliest produces the largest and most vigorous seedlings for transplanting.

Practically all the evidence from these experiments strongly supports the conclusion that western yellow pine spring-sown seed beds should not be shaded where artificial watering is possible, under the conditions in northern Idaho and western Montana. Duplicate experiments in two successive seasons, one of which was moist and favorable and the other was unusually hot and dry, uniformly show that the largest and most rapid germination, greatest survival, and best growth in the seed bed is obtained without shade, and that the unshaded seedlings make, on the whole, the best record in the transplant beds. There does not even seem to be any advantage in the temporary use of light shade during the hottest and driest part of the season, if water is frequently applied.

The optimum condition for the seedlings of this species appears to be full light and direct exposure to the sun at all times.

PROCEDURE

Two sets of experiments were included in this study, one in 1913 and one in 1914. The 1914 series was intended to check the results obtained in 1913 under different seasonal conditions, and to compare early spring sowing with the late spring sowing. The two seasons represented a wide contrast, 1913 being cool and moist, and 1914 unusually hot and dry. All plots were located in the Meadow nursery in connection with similar shading experiments with Douglas fir and western larch.

The original plan for the 1913 experiments included only two degrees of shade—one-quarter shade and no shade—since previous nursery experience had indicated that one-half shade was probably too heavy for an intolerant species like yellow pine. The no-shade and one-quarter shade plots were sown June 6, the late sowing being due to the unusually late season that year and unavoidable delays in getting the new nursery ready for sowing. At the end of the season, however, it was thought worth while to make a comparison between these two plots and another plot sown June 6 in a near-by bed which had been given one-half shade. This plot differed essentially from the other plots only in area, position in the bed, and degree of shade. Source of seed, date and method of sowing, depth of cover, character of soil, and other details of treatment were the same for all three plots.

One-half shade was included as a regular feature of the 1914 series, and three plots, one for each degree of shade, were sown May 6 in adjacent beds. Except for the degree of shade, all essential features of treatment were similar for the three plots.

Seed collected and extracted at the experiment station in the fall of 1912 was used for both the 1913 and 1914 experiments. All seed was sown broadcast and covered with clean sifted sand to a depth of

$\frac{1}{2}$ inch in 1913 and $\frac{3}{8}$ inch in 1914. Sand was spread by one-hand leveling boards and the depth was gauged by numerous toothpicks inserted to project $\frac{3}{8}$ inch above the sowing surface.

The one-quarter shade and no-shade plots in 1913 were 4 feet square and occupied one-third of the beds. They were sown with about 375 seed per square foot by distributing as evenly as possible an equal amount of seed over each plot, the seed being weighed out to centigrams. The one-half shade plot was 2 feet square in the $\frac{1}{2}$ -inch cover, broadcast plot, and was also sown at the rate of 375 seeds per square foot by weight.

In the 1914 tests all plots were $3\frac{1}{3}$ feet by 4 feet, the short dimension being caused by a vacant space left at the ends of each bed so that conditions surrounding the end plots might be similar to those surrounding the plots in the middle of the bed. Three equal quantities of seed, weighed out to centigrams so as to provide about 350 seeds per square foot, were evenly distributed over the plots.

To prevent damping-off, the 1914 plots were sprinkled with dilute sulphuric acid applied two-thirds before sowing and one-third after covering, at the rate of $\frac{3}{16}$ fluid ounce of acid to $1\frac{1}{2}$ pints of water per square foot. The 1913 plots were not sterilized.

Germination and survival counts were restricted to certain parts of each plot because of the limited time available for such work, except for the small one-half shade plot of 1913, which was counted completely. In the other 1913 plots a counting area 2 feet square was marked off in the center by pressing lath into the soil edgewise. In the 1914 plots two areas 1 foot square were used. These were centrally located about 1 foot from either side of the bed and marked by bent telephone wire laid on the surface and fastened by pegs.

Standard shade screens, made by nailing common lath to an outside frame of 2-inch strips at a distance of one lath width apart, were used to furnish one-half shade. One-quarter shade was provided by the use of lath sawed in half lengthwise, and spaced $1\frac{1}{2}$ full lath widths apart. Such a frame distributed the light and shade more uniformly than if full-width laths spaced 3 widths apart had been used. The no-shade plots received full light, except for a little shade along the edges of the south side and ends of each bed caused by the 2-inch bars of the protective frame and the $\frac{1}{2}$ -inch mesh wire of the screens.

Beds were laid out in an east-and-west direction so that the lath shadows would move from west to east. During both years shade frames were left on continuously from the time of sowing until the end of September.

Watering was carefully regulated in each season so as to give uniform treatment to all plots. With the nozzle set at a certain point, all beds were sprayed for the same number of minutes at each watering.

The stock raised in 1913 was transplanted May 1 and 2, 1914, and was divided into separate lots according to the degree of shade it had received. Part of the 1914 stock was transplanted in November, 1914, and was kept separate according to degree of shading and months of germination. The rest of this stock, separated only according to degree of shading, was transplanted April 26, 1915. All transplanting was done with a standard Yale board in trenches dug with a spade, and the work was uniform for all lots.

Counts of germination and survival were made about once a week after germination started during both seasons. At the end of the season a count of all living seedlings in the counting areas was made as a check on the total brought forward weekly. Also a final count of the total number of surviving plants outside the counting area was made to determine how closely the counted areas represented the rest of the plot. Survival counts of the transplants were made in the early summer and fall of 1915.

When seedlings were taken up for transplanting in the fall, a sufficient number from each plot in each bed to give a good average were selected arbitrarily, and measurements of length of main tap root and length of stem to tip of bud were taken. The plants were carefully dug to a depth of about 18 inches, and the soil was loosened from the roots with the fingers so as to bring out practically all of the main root system with the plant. In addition, the 1913 samples were washed and surface-dried and divided at the ground line, the two lots of roots and tops being weighed to centigrams. The 1914 samples were not weighed, but were measured to obtain the average length of leaf in the main top and the diameter of the stem at the ground line. In the fall of 1914 measurements were taken separately according to month of germination. Measurement of the length of root was omitted for the stock taken from the 1914 beds in the spring of 1915.

In the fall of 1915 each tenth transplant from each lot in the transplant bed was measured to determine the average height of stem and diameter at ground line.

At the time seedling stock was measured about five plants typical of those with average measurements were selected from each lot and pressed. Photographs were made of typical seedlings from each of the three degrees of shading and also of seedlings from the unshaded bed, to show the relation between month of germination and size of the plants.

DATA

The evidence brought out by the experiments is, briefly, as follows:

The unshaded bed in 1913 produced much the largest total germination for the season, one-quarter shade being 20 per cent less, and one-half shade 50 per cent less. The same marked relation is shown by the 1914 beds, although the decrease in germination in the shaded beds is proportionally not so great.

The rate of germination was most rapid in the unshaded beds in both years. In 1913 the ratio was 45 for no shade, 35 for one-quarter shade, and 20 for one-half shade. The relation in 1914 is shown graphically by the curves in Figure 3. The general form of the curves is the same, but the curve for the unshaded bed rises more rapidly than those for the shaded beds.

Percentage of germinated seedlings surviving at the end of the season is greatest in the unshaded beds in both years, both for the individual months of germination and for the plots as a whole. Expressed in percentage of plants germinated, the proportion in the 1913 beds was 94.6 per cent for no shade, 78.4 per cent for one-quarter shade, and 85.9 per cent for one-half shade. In the 1914

beds the percentage of germinated seedlings surviving was practically the same for no shade and one-quarter shade (85.3 and 85.7), but was distinctly less for one-half shade (78.5).

The total number of surviving plants at the end of the season, in the entire beds in 1913, including those in the germination-count plots, when expressed in terms of the percentage of seed sown, made a distinct showing in favor of no shade. The percentage for

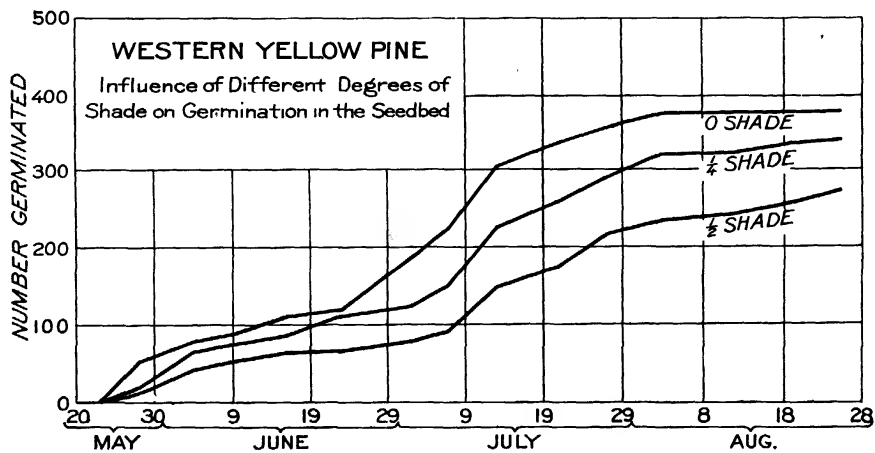


FIG. 3.—Weekly march of germination, 1914: Degree of shade

no shade was 64, for one-quarter shade next at 51 per cent, and one-half shade least with 48 per cent. Losses in the 1914 beds are shown in detail in Table V.

TABLE V:—Losses by months under different degrees of shade, 1914 beds

Month	Loss, no shade			Loss, 1/4 shade			Loss, 1/2 shade		
	Dead seedlings	Per-centage of germina-tion to date	Per-centage of season loss	Dead seedlings	Per-centage of germina-tion to date	Per-centage of season loss	Dead seedlings	Per-centage of germina-tion to date	Per-centage of season loss
June.....	23	12.2	58.9	18	6.1	46.1	12	15.4	20.0
July.....	14	3.7	35.9	17	3.4	43.6	22	9.3	36.7
August.....	1	0.3	2.6	1	0.2	2.6	9	3.2	15.0
September-October.....	1	0.3	2.6	3	0.6	7.7	17	5.9	28.3
Season loss.....	39	10.1	100.0	39	7.5	100.0	60	20.8	100.0

The largest and best developed plants were obtained from the unshaded bed, the second best from the one-half shaded bed, and the smallest from the one-quarter shaded bed.

The fact that there is a marked difference in size of seedlings according to month of germination, as shown in Figure 4, is an additional argument in favor of no shade. As the earliest germination occurs without shade it should naturally follow that a greater proportion of large, well-developed plants will be produced without shade than when either one-quarter or one-half shade is used.

For the 1913 beds, stock given one-half shade shows the greatest percentage of transplants alive both in the spring count (after one season) and the fall count (after two seasons), with one-quarter shade second and no shade last. The percentages were 95, 92, and 83, respectively. The better survival for the one-half shade stock possibly may be accounted for because of the lesser density of plants in the one-half shade plot, or because of some unknown variable in the transplant bed.

Survival of transplants from the 1914 beds given in Table VI corresponds in the spring count of 1915 with the development of the seedlings and shows 63 per cent for no shade, 61 for one-quarter shade, and 59 for one-half shade. Survival in the fall, in percentage of the number transplanted, was greatest for the one-quarter shade stock (56), with no shade next (52) and one-half shade lowest (47).

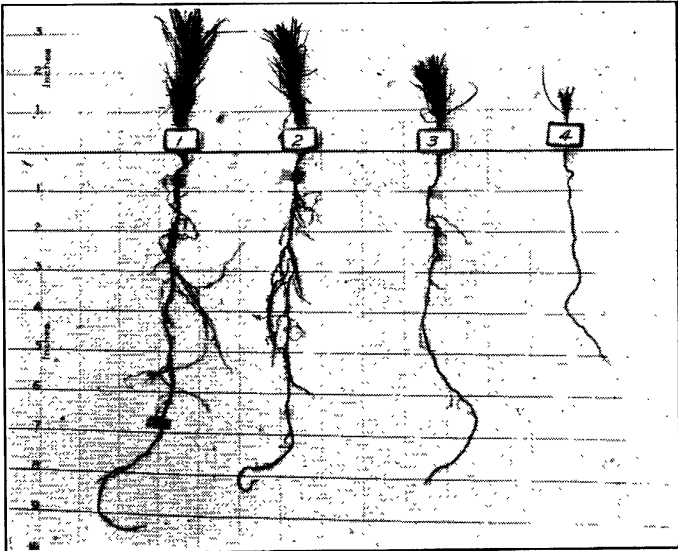


FIG. 4.—Effect of early germination on size of seedlings (*Pinus ponderosa*). Comparison, at end of season, of seedlings germinating in May (1), in June (2), in July (3), and in August (4)

TABLE VI.—Seedling measurements and transplant data by month of germination, fall transplanting, series of 1914

Degree of shade	Month of germination	Seedling measurements				Transplant data			
		Length root	Length stem	Length leaf	Diameter stem	Survival		Height stem	Diameter stem
						First spring	First fall		
		Inches	Inches	Inches	Mm.	Per cent	Per cent	Inches	Mm.
No shade-----	May-----	11.6	2.7	1.19	1.9	94.9	93.1	4.3	3.4
	June-----	10.2	2.4	1.07	1.8	75.8	62.8	3.6	3.0
	July-----	9.2	1.7	0.82	1.3	53.5	44.5	3.4	2.8
	August-----	5.4	1.1	0.48	1.2	10.0	0.0		
	Mixed-----					70.2	57.9	3.5	3.0
Weighted average----		9.1	1.7	0.89	1.5	63.1	51.7	3.7	3.0
One-quarter shade ----	May-----	9.5	2.1	0.90	1.5	92.8	91.0	3.2	2.8
	June-----	8.7	1.8	0.87	1.3	85.0	70.0	3.1	2.6
	July-----	8.1	1.6	0.78	1.2	63.7	54.8	2.8	2.0
	August-----	5.6	1.2	0.48	1.2	40.0	35.0	1.8	1.9
	Mixed-----					74.0	57.0	2.8	2.5
Weighted average----		7.9	1.6	0.75	1.3	60.8	56.5	2.7	2.4
One-half shade-----	May-----	10.5	1.9	0.99	1.4	97.8	97.0	3.4	2.6
	June-----	10.5	1.5	0.86	1.3	70.4	58.2	2.6	2.3
	July-----	8.7	1.4	0.71	1.2	70.4	45.4	2.8	2.2
	August-----	6.5	1.0	0.50	1.2	4.3	3.3	1.7	1.7
	Mixed-----					62.8	56.5	3.1	2.8
Weighted average----		9.0	1.4	0.86	1.2	58.9	47.3	2.7	2.3

The survival figures for the transplants are rather contradictory and seem to show that the influence of shading in the seed bed has at least partly disappeared and given place to other and more direct influences in the transplant bed itself. The fact that the shaded stock made a slightly better showing may indicate that shade helps to fit the plants to withstand the shock of transplanting. This does not seem reasonable, however, as the more probable effect of shade would be to make them less resistant to severe conditions.

The size of the transplants in the first test did not vary in accordance with the shade received, those receiving one-half shade being much the largest, and the others about equal. The unshaded transplants obtained next year were distinctly the largest, however, with one-quarter shade stock slightly larger than that from one-half shade.

AMOUNT OF WATER WITH OR WITHOUT CULTIVATION

SUMMARY

It was to obtain some definite information as a basis for the standardization of watering western yellow pine that the following experiment was undertaken, bringing out the effect of the different kinds of watering and cultivation upon the growth and development of the stock, first as seedlings and later as transplants.

It is evident that the cheapest method of watering is to water heavily at rather infrequent periods. If water is more difficult to obtain in large quantities, lighter and more frequent watering, with cultivation to conserve the moisture, might be most profitable. Or, if the watering became very expensive, it might pay better to cultivate frequently enough to maintain a good dust mulch and retain in the soil as much as possible of the natural rainfall.

While the combination of moderate watering with cultivation produced the best results in this experiment, the difference in favor of this treatment was comparatively small. In order to cultivate it was necessary to sow in drills. In the second section of this report it was demonstrated that there was a material saving of seed, labor, and space when seed was sown broadcast. The slightly increased growth of the plants due to cultivation would hardly justify the lack of economy due to drill sowing where plenty of water was available. The cost of cultivating between drills, which has to be done with great care in order to avoid injury to the plants, would be an additional argument against cultivation. Broadcast sowing, with rather heavy watering at frequent intervals sufficient to maintain an average water content of more than 50 per cent of dry weight, is therefore unquestionably the best method for use at large nurseries where an abundance of water under pressure is to be had.

In small ranger nurseries where water is scarce or it is inconvenient or expensive to apply it, very good results may be obtained in the western part of the northern Rocky Mountain region on moderately heavy moisture-retentive soil, by raising the stock in drills and cultivating the surface either without artificial watering or with a moderate amount of water applied at times of special need.

PROCEDURE

Beds in the Meadow nursery were carefully prepared for sowing by spading the surface and mixing in sharp sand to loosen the top soil. Seed was sown in drills June 6, 1913, the late sowing being due

to the unusually late season and unavoidable delay in getting the beds ready. About 375 seeds (as determined by weight) were used per drill, that number being sufficient to produce an estimated stand of 200 plants per drill four feet long. Drills were 3 inches apart. One-half inch of clean sand was used for covering seed.

The beds were given one-half shade from time of sowing to the end of the season, except when frames were removed for short periods to check damping-off. Protection from birds and rodents was provided by a Pettis seed-bed frame.

Counts of germination and loss were made about once a week from the time of first germination until September, with a final count in mid-October. Plants were left in the beds for two seasons and were counted again at the end of the second season.

All beds were watered equally during the period of heaviest germination up to August, in order to establish a fairly complete stand before differentiation in treatment began, since it was not desired to include in this study the effect of watering upon germination.

After the first week in August the different beds were given the following kinds of watering treatment:

- (1) No artificial watering was done, and the surface was cultivated every fourth day and as soon after every rain as the ground could be worked. A uniform dust mulch was maintained in this way.

- (2) Two quarts of water were applied per square foot every fourth day. Surface was cultivated after every rain and as soon after watering as the ground could be worked.

- (3) One quart of water was applied per square foot every other day. No cultivation was given.

The differentiation in treatment was delayed because of the slow germination due to the late sowing. Although the treatment was kept up during the last three weeks in August and throughout September, the period was not long enough to affect the plants materially. It was therefore decided to continue the experiment through a second season and to make the contrast between treatments great enough to cause differences in growth in spite of the tendency of the plants to adjust themselves to changed conditions.

The following different treatments were given the second season:

- (1) No artificial watering was done. Surface was cultivated as soon after every rain as the ground could be worked. It was also cultivated often enough between rains to maintain a dust mulch.

- (2) A moderate amount of water was given at the end of each week, the amount being regulated so that the sum of the rainfall and artificial water made a total of 0.75 inch per week. The water applied artificially was reduced to inches by timing the flow with the nozzle set at a certain point and measuring the cubic inches of water discharged per minute. The surface was cultivated after each watering and each rain.

- (3) The bed was heavily watered, enough water being applied to make a total, when combined with rainfall, of 0.75 inch for each one-half week. Watering was done on alternate Tuesdays and Wednesdays, as representing the middle of the week and again on Saturdays, thus given an interval of approximately one-half week between applications, not counting rains.

When the precipitation during the week amounted to more than 0.75 inch in the case of bed 2, or to more than 0.75 inch for the semi-weekly period in the case of bed 3, the total amount of precipitation was noted, but the surplus was not carried forward into the next period, as the amount of the surplus was the same for all beds.

During the growing season soil samples were taken regularly each week from each of the three beds, as follows: Surface inch, 2 to 6 inch core, 7 to 12 inch core, and 13 to 18 inch core. The moisture content of each core was determined.

About one-half of the stock was transplanted in November and one-half in April. In the fall, at the time of transplanting, measurements were taken of 100 plants to show length of root in inches, length of stem in inches, diameter of stem at ground line in millimeters, and length of leaf in inches.

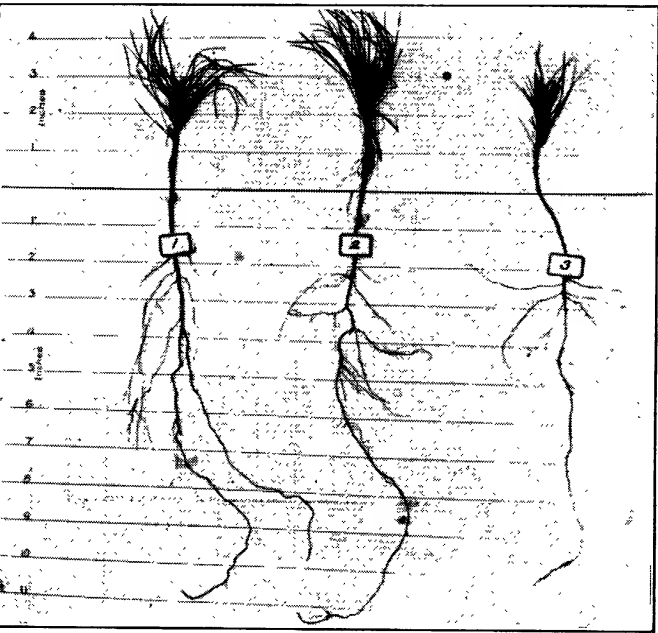


FIG. 5.—Watering versus cultivation. Average seedlings from three beds which received heavy watering only (1), watering and cultivation (2), and cultivation only (3)

A survival count was made in the early summer and in the fall of 1915, at which later time measurements of height and diameter of stem were made.

When the stock was transplanted, five seedlings, typical of average measurements, were selected from each bed and pressed. One of each of these lots of samples, photographed in order to illustrate the difference in development due to watering treatment, is shown in Figure 5.

DATA

Seed-bed germination and survival have little bearing on the study, because most of the germination was purposely allowed to take place before differentiation in watering treatment began. The relative merits of the three methods of treatment must, therefore, be judged by their effects upon the development of the seedlings and the growth and survival of the stock in the transplant beds. The essential records are included in Tables VII, VIII, and IX.

TABLE VII.—Water content of seed-bed soil under different methods of watering, second season, 1914

Method of treatment	Percentage of average moisture content				Maxi- mum at 1 to 6 inches	Mini- mum at 1 to 6 inches
	Surface inch	1 to 6 inch core	7 to 12 inch core	13 to 18 inch core		
Cultivation only	23.5	39.0	42.1	43.5	<i>Per cent</i> 54.3	<i>Per cent</i> 14.5
Cultivation and watering	33.4	54.1	57.8	54.7	69.0	40.4
Watering only	42.0	56.1	57.5	60.3	67.8	46.2

TABLE VIII.—*Survival, and growth of seedlings under different methods of watering, 1913-14*

Condition of seed			Survival of seed germinating				Measurements, second fall			
Method of treatment	Germination		First fall		Second fall		Root length	Stem length	Leaf length	Stem diameter
	Number	Per cent	Number	Per cent	Number	Per cent	Inches	Inches	Inches	Mm.
Cultivation only.....	759	63.2	509	67.1	331	43.6	11.7	2.3	1.9	1.8
Cultivation and watering.....	943	78.6	622	66.0	441	46.8	14.4	3.0	2.3	2.3
Watering only.....	753	63.2	484	64.3	298	39.6	13.6	2.8	2.1	2.1

TABLE IX.—*Survival and growth in the transplant bed under different methods of watering, 1915*

Season transplanted	Treatment	Survival of transplants		Measurements (fall)		
		Spring	Fall	Height	Diameter	Size factor *
		Per cent	Per cent	Inches	Mm.	
Fall.....	Cultivation only.....	95.4	82.0	3.5	3.2	5.60
	Cultivation and watering.....	91.8	82.5	3.7	3.2	5.92
	Watering only.....	90.2	78.6	3.4	3.1	5.27
Spring.....	Cultivation only.....	96.0	88.2	4.0	3.1	6.20
	Cultivation and watering.....	92.8	88.2	3.6	3.3	5.94
	Watering only.....	95.0	88.8	3.3	2.9	4.78

* One-half diameter multiplied by height.

The points brought out by the tables may be summarized as follows:
The largest plants were produced in the artificially watered beds. The beneficial effect of cultivation is clearly seen in the fact that the bed which was both watered and cultivated, even though it received only half as much water as the bed which was watered and not cultivated, produced noticeably larger plants than did the latter.
The soil moisture figures in Table VII show that the watered and cultivated bed had at all times practically as high a water content in the root zones as had the more heavily watered bed. The cultivating evidently helped to preserve moisture, but the greater growth in the cultivated bed must have been primarily due to the stirring of the soil rather than to the preservation of moisture. The exact effect of this stirring of the soil is not known, but it may have helped in aerating the soil and liberating plant food. One point indicated by the moisture-content data is that when the surface is cultivated, only half as much water is needed to maintain the soil moisture at a desirable point as when cultivation is not done.
The minimum moisture content figures show that soil moisture, even in the cultivated and unwatered bed, never approached a critical point. The hygroscopic moisture for similar soil in the vicinity is less than 4 per cent, and the lowest percentage reached was only 14.5.

Table VIII shows the measurements of the average 2-year-old seedlings from the three differently treated beds. The greater average size of the plants in the moderately watered and cultivated

bed could not have been due to more growing space, as there was a decidedly smaller number of plants in the other two beds throughout both years in the seed bed.

The stock from the moderately watered and cultivated bed maintained the lead attained in the seed bed and produced slightly larger and better developed transplants than that from the other beds. The most interesting point brought out by the transplants, however, as shown in Table IX, is the relative increase in size of the plants from the unwatered cultivated bed. In distinctly third place in the seed bed, as transplants they surpassed those from the heavily watered bed and almost attained first place, being practically as large as plants from the watered, cultivated bed. This seems to indicate that small size in the seed bed is not necessarily an undesirable feature for transplanting, if the plants are hardy.

The survival figures in the transplant bed show no clearly defined distinctions among the different treatments.

CONCLUSIONS

The following conclusions were reached from the results of the experiments:

(1) When clean, sharp sand is used and plenty of water is available, yellow pine seed in spring-sown beds should be covered to a depth of from $\frac{1}{4}$ to $\frac{3}{8}$ inch.

(2) Where an abundance of water can be applied, highest germination and survival in the seed bed and best development of the plants can be obtained if seed is sown broadcast rather than in drills. Broadcast sowing is also more economical of labor and space.

(3) Shade is not only not necessary for western yellow pine seedlings in spring-sown beds in this region, but has a distinctly undesirable effect on the amount and rate of germination, the amount of survival, and the development of the plants. Shade should not be used at any time. The optimum condition for seedlings of this species seems to be direct exposure to the sun.

(4) In large nurseries where plenty of water can be applied, seed should be sown broadcast and the beds watered rather heavily at intervals frequent enough to maintain an average soil-water content of more than 50 per cent of dry weight.

(5) In small ranger nurseries where water is scarce or it is too inconvenient or too expensive to apply, very good results may be obtained in this region on moderately heavy moisture-retentive soil by sowing in drills and cultivating between the rows, either without artificial watering or with a moderate amount of water applied at times of special need.

YIELD CAPACITIES OF THE PURE YELLOW PINE TYPE ON THE EAST SLOPE OF THE SIERRA NEVADA MOUNTAINS IN CALIFORNIA¹

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INTRODUCTION

East of the Sierra Nevada Mountains in California the predominating and most important forest type is western yellow pine (*Pinus ponderosa*). This type is represented by pure stands extending over large areas, in contrast with the characteristic mixed forest in which yellow pine is commonly found west of the Sierras and on the coast ranges. The lower precipitation on the east slope, the poorer soils, and shorter growing season, by creating conditions unsuitable for the more exacting sugar pine (*Pinus lambertiana*) and the firs, account for these pure stands and also for a generally lower rate of growth than is found elsewhere in the pine region. These conditions prevail over a considerable strip of country, and the east-slope pine in California is a part of merchantable stands of pure or nearly pure yellow pine ranging from western Washington and Oregon down to Arizona and New Mexico, all in many respects similar.²

Cutting in these western yellow pine forests is proceeding rapidly, both on national and private forest lands, and it is therefore important with regard to the possibility of obtaining new timber crops to study the productive capacity of the forest soils and to determine the amount of wood which can be produced with careful forest management. An immediate problem in practicing forestry on large areas of virgin forest is to develop a method for determining from the forest itself the productive capacity of a given area; for yellow pine stands vary tremendously in this region.

Yellow pine stands have been so little investigated that the technique of yield studies as applied to this species is itself a subject for investigation. The present study, which covered a large area in the eastern half of the Lassen National Forest, aimed not only to present preliminary information on yields of different qualities of forest land and on the probable rotations that will be used in growing the second crop of timber, but also to develop methods of procedure which might be applicable in similar investigations in other regions.³

FIELD METHODS

It soon became apparent that the traditional field methods for studying forest yields would have to be modified in order to meet the rather unusual conditions which were encountered in these stands.

¹ Received for publication May 26, 1925; issued December, 1925.

² The definition of a pure stand, which seems well established by usage, is one in which the dominant species forms 80 per cent or more of the total stand. This definition has been accepted and used in the present study.

³ Assistance in field work was given by Duncan Dunning, E. N. Munns, and A. E. Wieslander; in office work by Miss D. H. Vinther and Vance S. Brown. The project here reported was carried out in 1919.

In the first place there was practically no true second growth in this locality. Almost the entire area was virgin forest in which no cutting at all had been done. Secondly, only one mill was cutting in the area, so that the opportunities for laying out plots and for obtaining taper measurements were exceedingly limited. Further, no volume tables were in existence which would be suitable for immature yellow pine and white fir (*Abies concolor*), that is, for trees up to about 150 years old; and therefore as an accompaniment to the main study it was clearly necessary to collect data for the construction of such tables.

The virtual absence of true second growth made it compulsory to select even-aged groups of the younger age classes growing within the virgin forest, such as commonly occur where fire or insects have created small openings within the main stand and where reproduction has come in and made a fully stocked stand. These groups by no means form an ideal substitute for true second growth, for it is quite certain that the mature trees surrounding them have influenced the growth of the younger classes, probably to a considerable extent. A further disadvantage is entailed by the fact that these even-aged groups within the virgin forest are commonly rather small and it is often almost impossible to lay out an isolation strip surrounding the plots. In the majority of cases, in order to obtain plots of a satisfactory size, it was necessary to include practically the entire group of young trees, every effort being made to keep the boundary lines sufficiently far out from the edge of the crowns so that too high an apparent yield would not be secured. With the great majority of the plots, also, the usual rectangular form could not be maintained, for the boundary lines had to conform to the shape of the plot on the ground.

The absence of cuttings and the undesirability of slashing down too many trees, together with the fact that cutting of trees on every plot would have slowed down the work very greatly, made it necessary to obtain age determinations by means of the increment borer. Cores were taken at breast height on several trees on each plot and the usual stump analysis measurements made on these cores. In selecting trees for boring, both large and small trees on each plot were taken, to be certain that the plot was even-aged and not of several ages.

In order to determine the number of years required by the trees to reach breast height, seedling analyses were made on or near practically every plot measured. The seedlings were selected, as far as possible, from those growing in small openings in the stand and apparently were dominant. Toward the end of the work, when an opportunity arose to lay out plots in the true second growth itself, similar seedling analyses were made in large openings created by clear cutting in the virgin stand. In this way a very good idea was obtained of the difference in rate of seedling growth in the virgin stand, where unquestionably the seedlings are badly oppressed, and in the open under conditions such as would follow cutting of the old timber.

In the determination of heights it was decided to measure only one or two trees in each of the four dominance classes (dominant, codominant, intermediate, and suppressed), rather than to attempt

a measurement of each individual tree on the plot. By this method a height-diameter curve for each plot or for each group of plots can be drawn, and in computing volumes the height indicated by the curve can be used with very little chance of serious error. Computations on several of the plots laid out by Gallaher⁴ in west side pure yellow pine showed an error of from 1 to 2 per cent in using this method, the values where every tree was measured tending to run slightly higher than where the height-diameter curve was used. This short-cut method was felt to be fully justified, however, by the rate of speed which it made possible and because, so far as can be determined, any error introduced is likely to be on the side of conservatism.

Wherever possible, dominant and codominant trees on the plots were climbed and taper measurements taken, these trees later being used for the construction of a volume table. On part of the plots, also, sample trees were felled and complete stem analyses made. Occasionally wind-thrown trees were also measured.

Work was carried on from six camps well scattered over the area, a radius of several miles being covered from each. Thus it is believed that a sufficient portion of the entire area was studied to be representative of the entire range of conditions in yellow pine stands.

In all, 175 plots were measured: 132 pure yellow pine, 22 pure white fir, and 21 mixed stands. The site qualities as measured by mature heights ranged from about 170 feet to as low as 80 feet, thus practically embracing the entire range of site quality on the east side of the Sierras. The average plot was almost exactly one-fourth acre in area, individual plots varying from one-twentieth of an acre to nearly an acre in extent. For the construction of yellow pine volume tables approximately 150 trees were taken. About 25 white fir also were measured.

It can be seen from the preceding discussion that the field methods adopted differed in some respects from the traditional textbook methods for studying yield, a situation made necessary by limitations of the forest itself and of men and money to carry out the work.

OFFICE METHODS

The office methods used were in the main those established by custom for the construction of yield tables. As a preliminary step, of course, the tree-volume data had to be worked up and tables constructed. The first question encountered was the selection of a log rule on which to base the tables. The Scribner Decimal C rule, which is in current use in the Forest Service, is obviously unsuited for the study of future yields because of the large overrun of material sawed out as against the values from the rule. The information desired was the number of board feet of lumber per acre that could be obtained, not how many thousand feet the log scale would estimate. The Clark International rule was therefore selected because of its close approximation to mill scale. In determining on a standard of utilization on which the yield tables should be based, many individuals were consulted. The general consensus of opinion designated 8 inches in diameter at breast height as the smallest tree to be considered, and

⁴ GALLAHER, W. H. SECOND GROWTH YELLOW PINE. Forestry Quart. 11:531-536, illus. 1913.

a 5-inch top as a cutting limit entirely within the bounds of possibility. Incidentally, these were the standards accepted by Gallaher for the west slope of the Sierras.

In the actual construction of the volume tables the data were worked up by three different methods, i. e., the conventional method, the frustum form factor, and the alignment method. The tables were then checked against the trees from which they were constructed and against each other, and it was found that the values derived by the different methods were generally exceedingly close, and that by each method the values from the tables checked well against the trees. In the same way cubic-foot tables were made and the board-foot tables checked against the cubic-foot tables, a conversion factor being used. Although it is recognized that the basis of data is rather small for construction of final volume tables for immature trees under east slope conditions, comparison of the values with those in the current tables indicates that the latter would be less suitable.

The values for a given total height and diameter class are higher on the east slope than on the west slope, using Gallaher's volume tables for the latter set of conditions. This is because the more rapid taper in the top of the east slope trees gives them a shorter top above the 5-inch merchantable limit than in the west slope trees. With trees of equal total height, those on the east slope thus have a greater merchantable length. If the tables were worked up on the basis of merchantable height instead of total height, the indications are that values would be higher for west slope than for east slope trees.

SITE CLASSIFICATION

A little consideration of the main problem, that is, the determination of the yield capacities of large areas of virgin forests, makes it clear that yield tables to be readily usable must be tied into some index which can be measured without difficulty. The principle that height of dominant trees at a given age or at maturity is a reasonably correct expression of differences in yield, or site quality, is so thoroughly established by both American and European experience that no objections could be seen to its adoption here, and consequently each plot was referred to the average height of dominant mature trees adjacent to the plot, usually four or five trees being measured to obtain a correct figure. One can readily see that this procedure makes possible at once an approximately correct site classification of the area where timber reconnaissance has been made. The original timber tally sheets show the occurrence of the mature timber by merchantable heights, and one readily picks out on the sheets the average maximum heights on a given forty. Then, after these heights have been entered on a base map, it is also easy to convert merchantable height to total height by adding the length of top above the merchantable limit used in the volume tables.

The concensus of opinion among foresters in this country seems to be that five site classes are sufficient, some writers, indeed, favoring three. After considerable discussion and study of the question it was decided to adopt the five-site quality classification and to use a difference of 20 feet in mature height as the interval between classes. This interval appears to be fairly well agreed upon and also has the great virtue of fitting the conditions in the forest. That is, in pure

yellow pine on the east slope the shortest timber is about 70 to 80 feet high, and the tallest about 170 feet, though very rarely stands nearly 200 feet high are encountered. On the particular area studied there are several fairly definite mature heights. Near the west side of Eagle Lake, for example, there is a large body of timber about 80 feet in height. On the easterly slopes in the same region, stands of 100 feet are found, and in the vicinity of Bogard Flat and on most of the buttes on the area, 120 feet is the common height of the virgin stand. The values finally selected therefore are: Site 1, over 150 feet; site 2, from 131 to 150 feet; site 3, from 111 to 130 feet; site 4, from 91 to 110 feet; site 5, from 71 to 90 feet. It is immaterial for this study whether this classification is the one finally determined upon for the region or whether it meets with the general approval of the profession; the data are such that any other better definition can be readily accepted, and the data tied in with it.

In accordance with this plan of site classification, the individual plots were sorted by age, 10-year age classes being used, and by site quality (Table I). The following data were computed for each individual plot and later plotted and curved on the basis of age classes: (1) Basal area per acre; (2) height of dominant trees; (3) diameter growth of dominant, intermediate, and suppressed trees; (4) trees per acre; (5) volume in board feet per acre; (6) volume in cubic feet per acre (Table II).

TABLE I.—Distribution of plots in east slope yellow pine type by age and site ^a

Age at breast height (years)	Distribution of plots						Age at breast height (years)	Distribution of plots					
	Total	Site 1	Site 2	Site 3	Site 4	Site 5		Total	Site 1	Site 2	Site 3	Site 4	Site 5
50.....	14	-----	7	5	2	-----	140.....	5	1	1	1	2	-----
60.....	11	1	5	5	-----	-----	150.....	4	-----	-----	1	2	1
70.....	8	1	-----	7	-----	-----	160.....	6	-----	1	4	1	-----
80.....	10	-----	1	4	3	2	170.....	3	-----	-----	-----	1	2
90.....	11	1	1	7	2	-----	180.....	3	-----	-----	3	-----	-----
100.....	10	-----	-----	6	2	2	190.....	1	-----	-----	1	-----	-----
110.....	9	-----	4	2	3	-----	240.....	1	-----	-----	-----	-----	1
120.....	22	3	3	12	2	2	Total.....	132	8	27	66	21	10
130.....	14	1	4	8	1	-----							

^a Twenty-two plots of pure white fir and 21 plots of mixed pine and fir were also obtained.

TABLE II.—Yield of pure yellow pine, Lassen National Forest, Calif.

[Clark International rule, 1/8-inch kerf; values curved]
SITE 1^a

Total age ^b (years)	Number of trees per acre	Basal area per acre	Height of dominant	Average diameter at breast height	Volume per acre		Periodic annual growth	Mean annual growth	Board-feet per cubic foot
		Sq. ft.	Feet	Inches	M. bd. ft.	Cu. ft.	M. bd. ft.	M. bd. ft.	
60.....	356	168	53.0	9.3	3.5	2,320	0.35	0.06	1.51
70.....	251	197	64.0	12.0	13.5	4,300	1.00	.19	3.14
80.....	202	225	75.0	14.3	27.5	6,480	1.40	.34	4.24
90.....	174	252	86.0	16.3	43.5	8,500	1.60	.48	5.12
100.....	158	276	95.0	17.9	58.5	10,250	1.50	.58	5.71
110.....	146	297	103.0	19.3	67.5	11,680	.90	.61	5.78
120.....	138	314	109.0	20.4	74.0	12,800	.65	.62	5.78
130.....	134	328	113.5	21.2	79.0	13,650	.50	.61	5.79
140.....	133	342	117.0	21.7	83.0	14,350	.40	.59	5.78
150.....	132	353	120.5	22.1	86.8	14,950	.38	.58	5.81

^a Mature heights, 151+ feet; yellow pine 80+ per cent; basis, 8 plots.
^b Age at breast height is 20 years less than total age.

TABLE II.—Yield of pure yellow pine, Lossen National Forest, Calif—Continued

SITE 2^c

Total age (years)	Number of trees per acre	Basal area per acre	Height of domi- nant	Average diameter at breast height	Volume per acre		Periodic annual growth	Mean annual growth	Board- feet per cubic foot ^e
		<i>Sq. ft.</i>	<i>Feet</i>	<i>Inches</i>	<i>M. bd.-ft.</i>	<i>Cu. ft.</i>	<i>M. bd.-ft.</i>	<i>M. bd.-ft.</i>	
60.....	417	142	41.0	8.0	1.0	1,060	0.10	0.02	0.94
70.....	280	165	51.0	10.4	8.0	2,660	.70	.11	3.01
80.....	222	189	61.0	12.5	17.0	4,260	.90	.21	3.99
90.....	196	215	71.0	14.2	27.0	6,030	1.00	.30	4.48
100.....	177	235	81.0	15.6	38.0	7,800	1.10	.38	4.87
110.....	164	255	91.0	16.9	48.0	9,110	1.00	.44	5.27
120.....	156	270	97.0	17.8	56.0	10,190	.80	.47	5.50
130.....	148	283	100.0	18.7	61.0	11,050	.50	.47	5.52
140.....	148	295	103.5	19.1	65.0	11,730	.40	.46	5.54
150.....	147	305	107.0	19.5	68.5	12,300	.35	.46	5.57

SITE 3^d

Total age (years)	Number of trees per acre	Basal area per acre	Height of domi- nant	Average diameter at breast height	Volume per acre		Periodic annual growth	Mean annual growth	Board- feet per cubic foot ^e
		<i>Sq. ft.</i>	<i>Feet</i>	<i>Inches</i>	<i>M. bd.-ft.</i>	<i>Cu. ft.</i>	<i>M. bd.-ft.</i>	<i>M. bd.-ft.</i>	
60.....	450	124	34.0	7.1	-----	-----	-----	-----	-----
70.....	331	143	42.0	8.9	3.0	1,180	0.3	0.04	2.54
80.....	273	164	52.0	10.5	9.0	2,600	.6	.11	3.46
90.....	229	183	62.0	12.1	17.3	3,900	.83	.19	4.44
100.....	209	202	71.0	13.3	25.7	5,400	.84	.26	4.76
110.....	191	219	81.0	14.5	34.0	7,000	.83	.31	4.86
120.....	178	230	85.0	15.4	40.7	7,970	.67	.34	5.11
130.....	167	237	88.0	16.1	45.0	8,600	.43	.35	5.23
140.....	162	243	91.0	16.6	47.7	9,100	.27	.34	5.24
150.....	158	249	93.0	17.0	50.5	9,570	.28	.34	5.28

SITE 4^e

Total age (years)	Number of trees per acre	Basal area per acre	Height of domi- nant	Average diameter at breast height	Volume per acre		Periodic annual growth	Mean annual growth	Board- feet per cubic foot ^e
		<i>Sq. ft.</i>	<i>Feet</i>	<i>Inches</i>	<i>M. bd.-ft.</i>	<i>Cu. ft.</i>	<i>M. bd.-ft.</i>	<i>M. bd.-ft.</i>	
60.....	526	100	28.0	5.9	-----	-----	-----	-----	-----
70.....	395	118	36.0	7.4	0.5	450	-----	0.007	1.11
80.....	335	135	43.0	8.6	4.0	1,600	0.35	.05	2.50
90.....	296	152	52.0	9.7	8.4	2,650	.44	.09	3.17
100.....	270	169	60.0	10.7	13.7	3,750	.53	.14	3.85
110.....	247	182	68.0	11.6	20.2	4,800	.65	.18	4.21
120.....	221	191	73.0	12.6	26.0	5,570	.58	.22	4.67
130.....	205	200	76.5	13.4	30.0	6,070	.40	.23	4.94
140.....	189	205	79.0	14.1	33.0	6,500	.30	.24	5.08
150.....	177	209	81.0	14.7	35.8	6,830	.28	.24	5.24

SITE 5^f

Total age (years)	Number of trees per acre	Basal area per acre	Height of domi- nant	Average diameter at breast height	Volume per acre		Periodic annual growth	Mean annual growth	Board- feet per cubic foot ^e
		<i>Sq. ft.</i>	<i>Feet</i>	<i>Inches</i>	<i>M. bd.-ft.</i>	<i>Cu. ft.</i>	<i>M. bd.-ft.</i>	<i>M. bd.-ft.</i>	
60.....	727	81	21.0	4.5	-----	-----	-----	-----	-----
70.....	531	94	28.0	5.7	-----	-----	-----	-----	-----
80.....	429	108	35.0	6.8	1.0	850	0.1	0.012	1.18
90.....	375	121	42.5	7.7	3.5	1,700	.25	.04	2.06
100.....	337	133	50.0	8.5	6.5	2,400	.30	.06	2.71
110.....	296	143	56.5	9.4	10.2	3,050	.37	.09	3.34
120.....	273	152	61.0	10.1	14.5	3,550	.43	.12	4.08
130.....	252	160	63.0	10.8	18.3	3,920	.38	.14	4.67
140.....	238	166	64.5	11.3	21.5	4,280	.32	.15	5.02
150.....	229	171	66.0	11.7	24.2	4,600	.27	.16	5.26

^c Mature heights, 131 to 150 feet; yellow pine 80+ per cent; basis, 27 plots.^d Mature heights, 111 to 130 feet; yellow pine 80+ per cent; basis, 66 plots.^e Mature heights, 91 to 110 feet; yellow pine 80+ per cent; basis, 21 plots.^f Mature heights, 71 to 90 feet; yellow pine 80+ per cent; basis, 10 plots.

The site 3 quality class was the one in which most of the work was done, 66 out of a total of 132 plots falling in that class. (See Table I.) Site qualities 2 and 4 also had a very fair representation of plots, but the values in basal area per acre and volume per acre for sites 1 and 5 were very poorly defined, and the curves are drawn in part by inference.

INDICATIONS OF THE DATA

It will be seen, considering first the chart for board-foot volume (fig. 1), that the curves flatten off very decidedly at about 90 to 100 years of age at breast height; in other words, that that age represents the culmination of current annual increment. A little study of the other charts will perhaps explain why volume growth falls off at 100 years. It is seen first (fig. 2) that basal area per acre also drops considerably at 100 years, and that height growth (figs. 3 and 4) culminates at that age, this curve especially showing a very sharp flattening. In diameter growth (fig. 5) and basal area growth of individual trees it is rather more difficult to say confidently that 100 years represents the culmination. Both of these curves show a very gradual flattening and a study of them alone would probably be of little assistance in determining rotation.

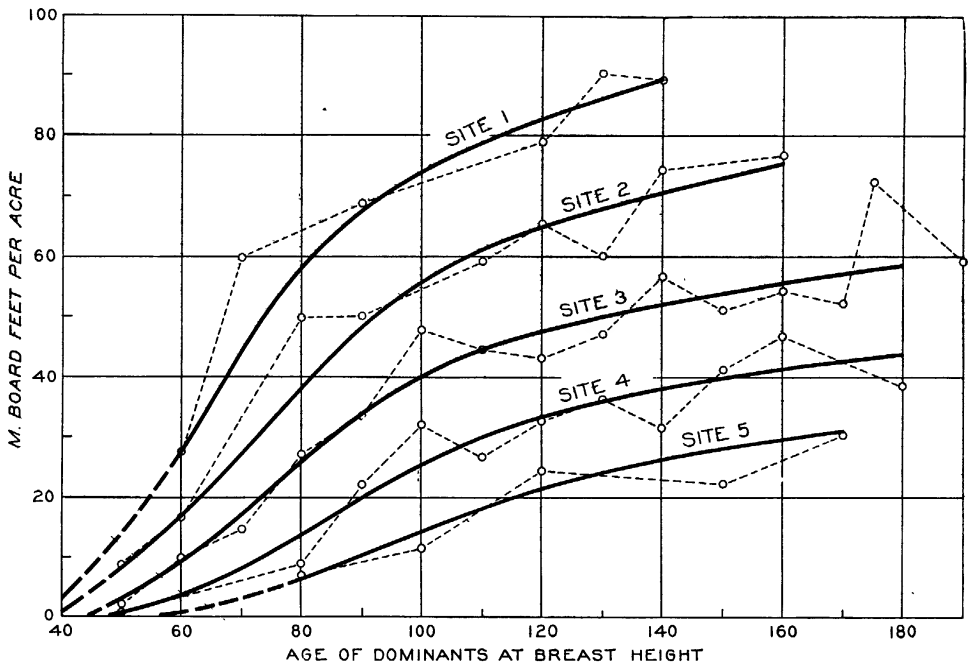


FIG. 1.—Yield per acre on age, board feet

The curves in Figure 6 showing trees per acre are unusually interesting. The upper curve gives total number of trees 4 inches in diameter up, while the lower curve shows trees per acre of merchantable size; that is, 8 inches and over. The latter curve indicates a rapid increase in the number of these trees per acre up to 80 years, and then a more gradual decrease in number up to the highest age reached by these studies. In other words, what evidently takes place in nature is that stands at 80 to 100 years reach a period of development when suppression, and perhaps other factors, begin to reduce the number of trees 8 inches and over more rapidly than smaller trees grow into that class. These curves thus show why and how volume growth begins to fall off after about 100 years age at breast height. Apparently height growth, total basal area growth, diameter growth, and number of trees per acre all tend to culminate at about

the same age, and obviously volume growth, which is a complex of these four factors plus form factor, must also culminate.

An examination of the volume curves (figs. 1 and 7) and of periodic annual increment (fig. 8) indicates that the better the site the earlier is the culmination of periodic annual increment. This seems to be in line with European and American observations.

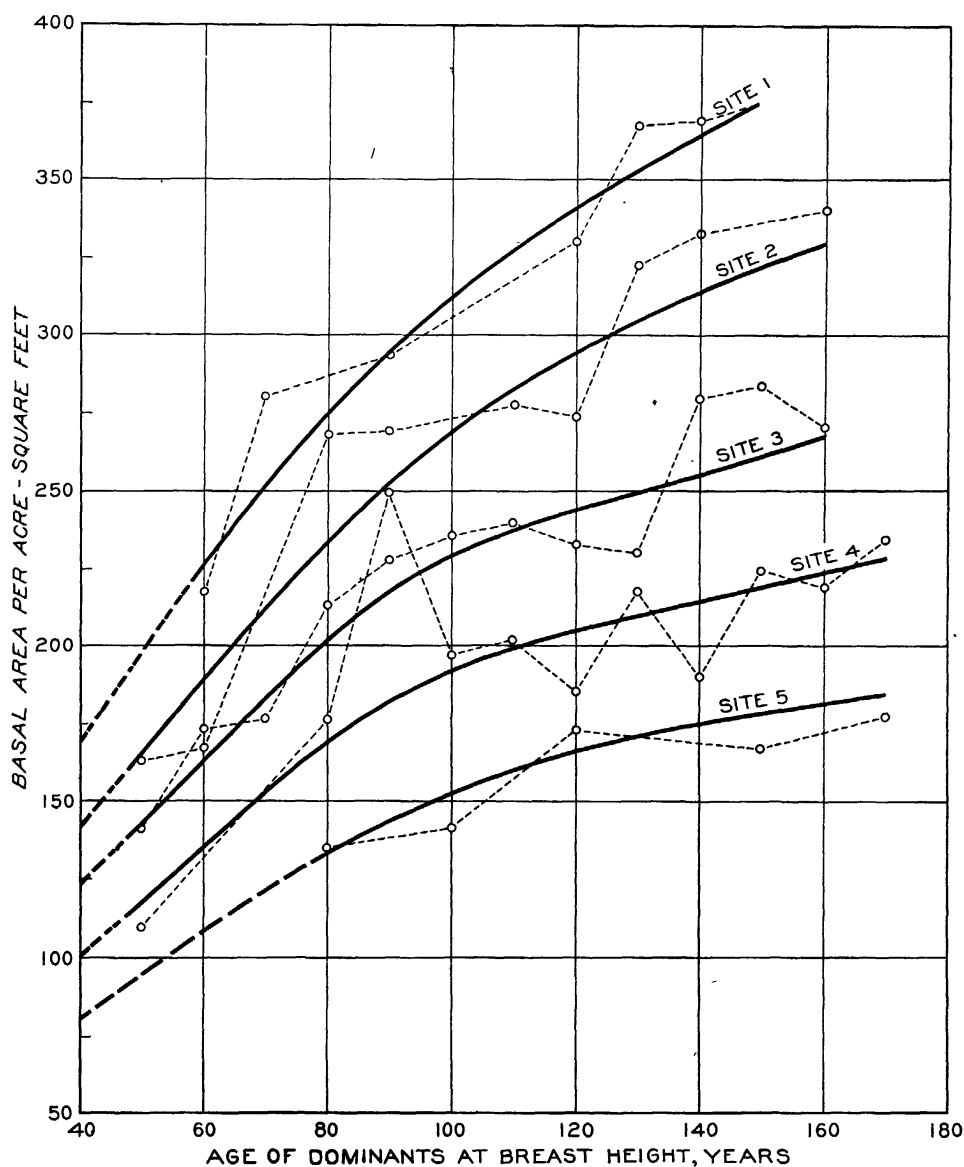


FIG. 2.—Basal area per acre on age

A study of the basal area curve (fig. 2) shows about equal intervals between sites, whereas in yield the interval between sites increases from poor to good sites. As far as a look into published data has shown, these tendencies seem to be in harmony with the results of more exhaustive work elsewhere. Of course these apparent agreements between the present study and other work do not necessarily prove anything. They may perhaps indicate that the method

of site classification adopted is not too bad, and that the values derived are at least relatively correct.

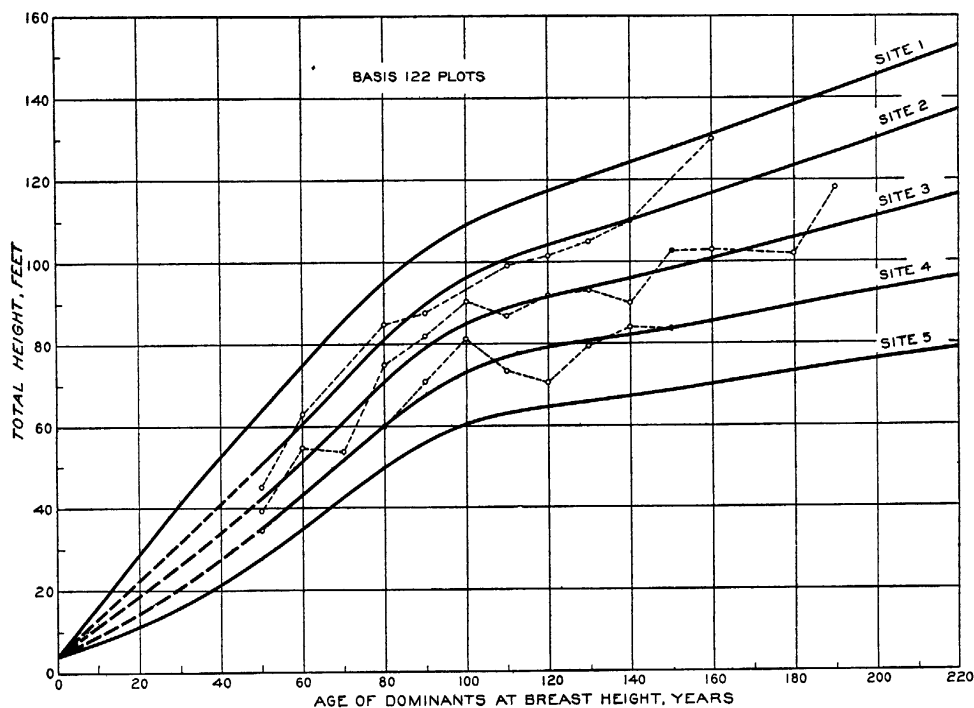


FIG. 3.—Heights of dominants on age

An attempt to check results by means of the “site factor” advocated by Hanzlik ⁵ in Oregon and Washington was not particularly satisfactory. Whereas he apparently found equal intervals between

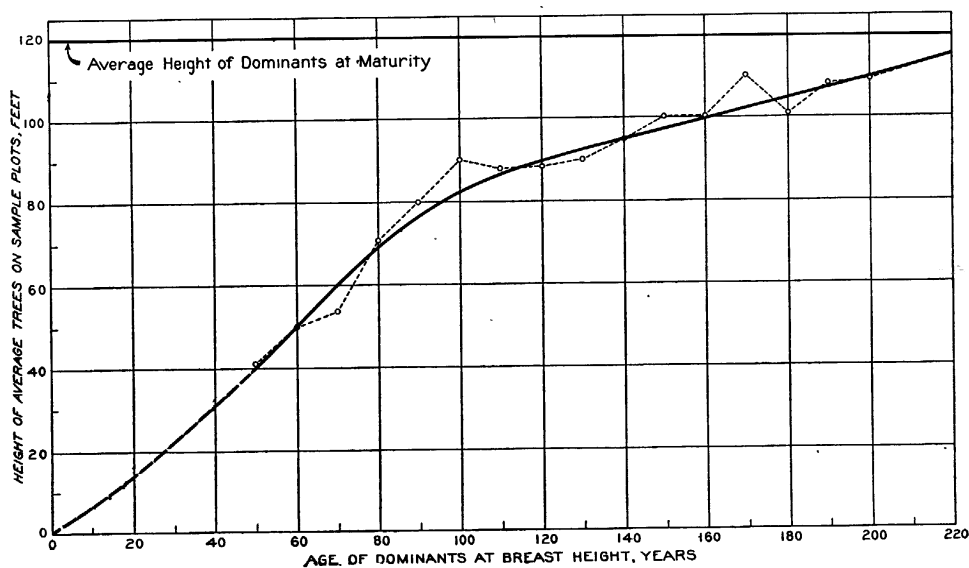


FIG. 4.—Relation of height of average trees to mature height of dominants on age of dominants

sites, figures in the present study show the factors varying in the same way as to yield, i. e., increasing from poor to good sites.

⁵ HANZLIK, E. J. THE DETERMINATION OF SITE QUALITIES FOR EVEN-AGED STANDS BY MEANS OF A SITE FACTOR. *Proc. Soc. Amer. Foresters* 9: 229-234. 1914.

As far as field application is concerned, the site factor method seems to have about the same deficiencies as the Baur method of site

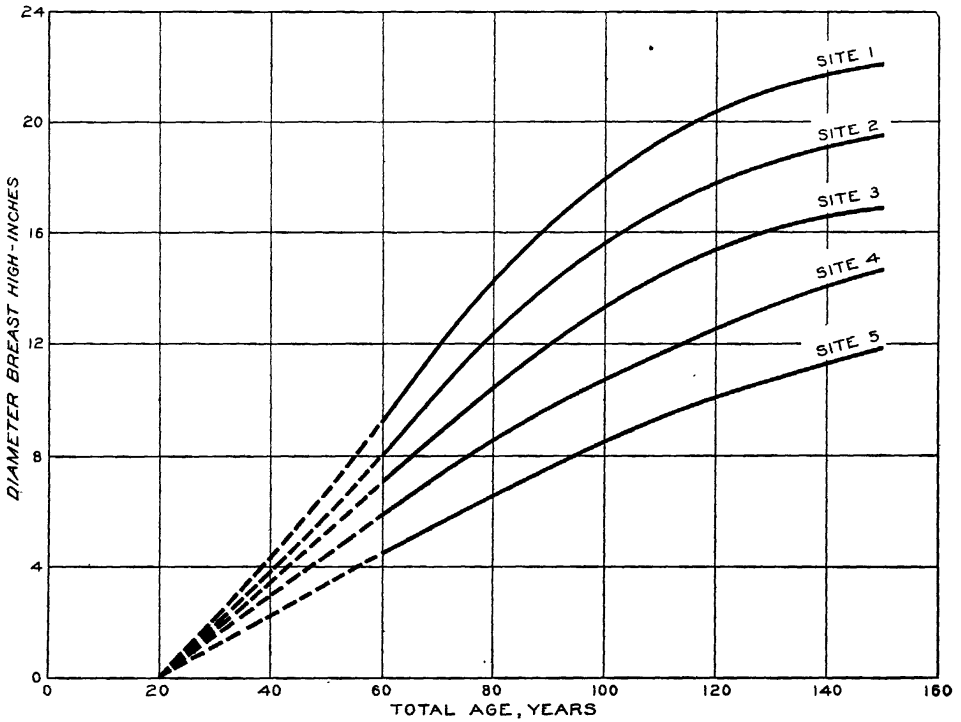


FIG. 5.—Diameter of average tree on age

determination; that is, both are primarily adapted to use in second-growth stands, and depend on measurement of plots for determination of either basal area per acre or volume per acre. In neither case is

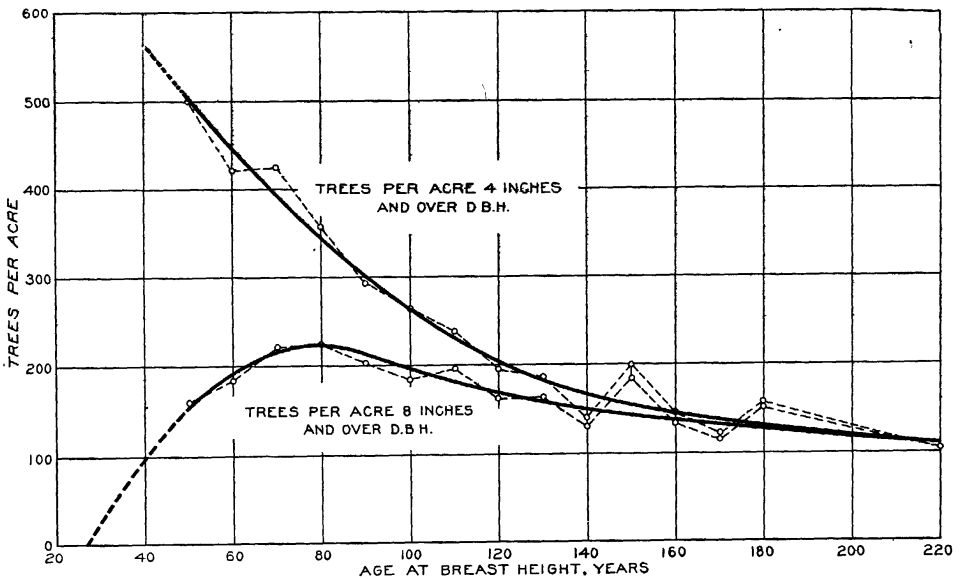


FIG. 6.—Average number of trees per acre on age

there any way of applying the results to areas of virgin forest, which seems to be the most important problem we have just now.

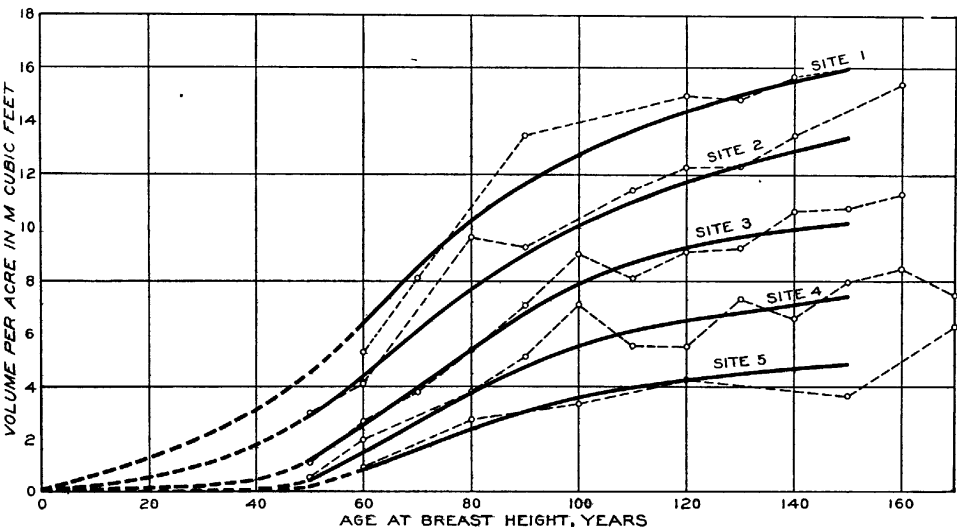


FIG. 7.—Yield in cubic feet per acre on age, bark included

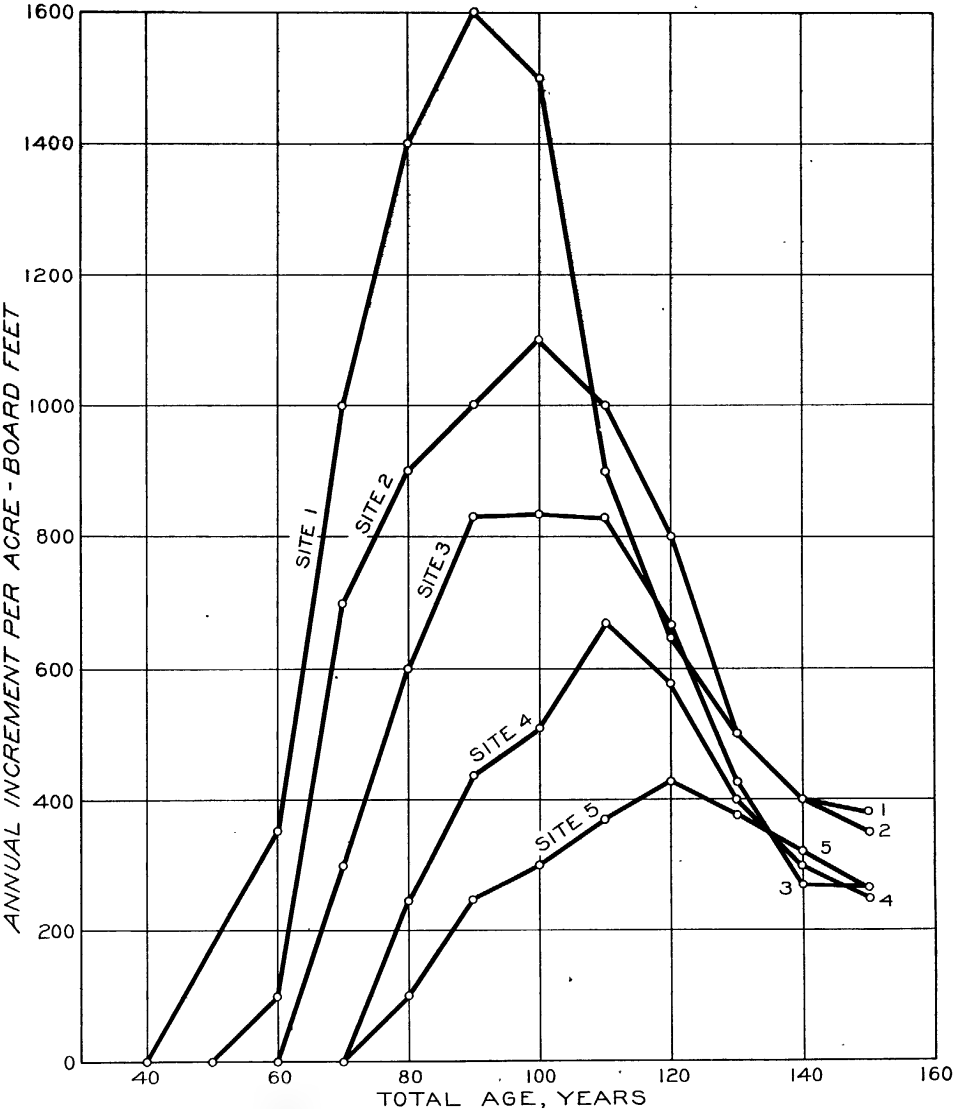


FIG. 8 — Periodic annual increment per acre, board feet

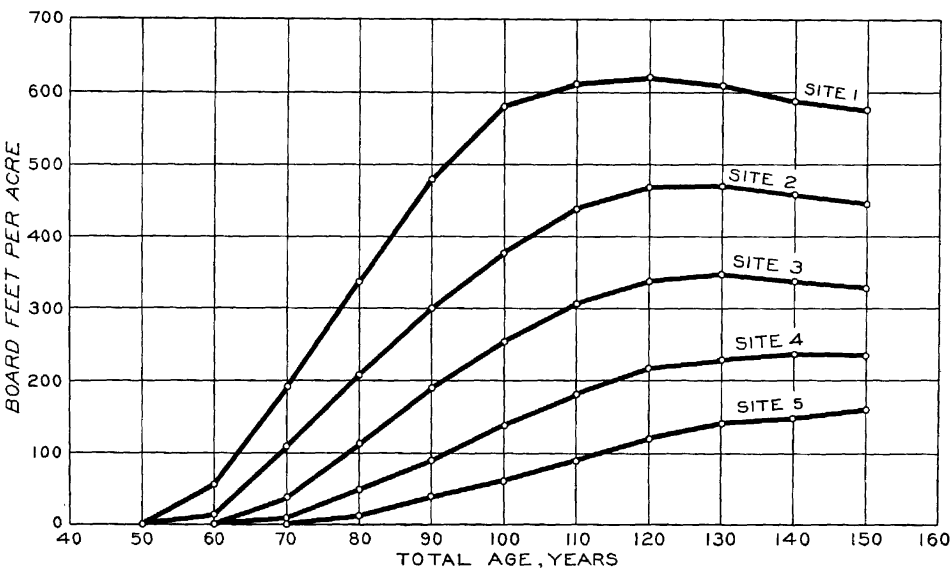


FIG. 9.—Mean annual increment per acre, board feet

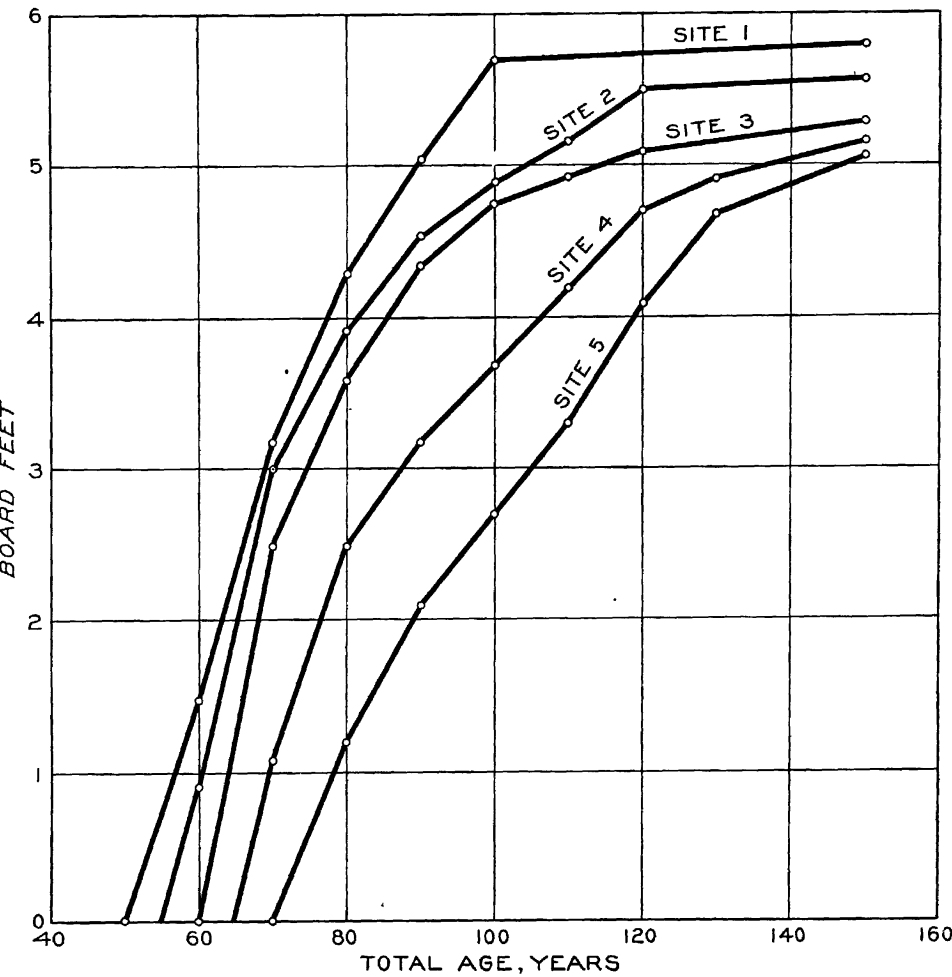


FIG. 10.—Board feet per cubic foot for the entire stand over 8 inches in diameter at breast height

DETERMINATION OF ROTATION

Various methods have been used for determination of rotation age and it is not the intention here to discuss these further. As a matter of pure silviculture the culmination of mean annual increment is probably the most generally recognized criterion. An examination of Figure 9 will show that mean annual increment reaches its highest point at an age of 120 to 130 years, after which it remains at practically the same level for 20 to 30 years before beginning to fall. The only excuse for extending the cutting age much beyond the culmination would be on the ground of quality increment, but where this is not important a rotation of 120 years at the outside is ample.

Figure 10, showing the board-foot cubic-foot ratio for the entire stand, also points to the conclusion that 120 years is about the proper cutting age. Up to that period in the stand's development on most of the sites the yield in board feet per cubic foot increases rapidly; beyond that point the yield is either practically constant or rises but slowly.

FACTORS OF CONSERVATISM

In the tables there are several elements which may be called factors of conservatism, which deserve discussion. The first of these is the allowance of 20 years for seedlings to reach breast height. As has already been explained, this will no doubt be lowered 5 to 10 years provided cutting is heavy, which will very likely result in a somewhat shortened rotation or a higher yield per acre on the same rotation of 120 years.

The second factor is the undoubted influence of the mature timber on the growth and yield of the plots. There is no way at present of measuring the influence of this factor, since practically no plots of the 50 and 60 year age classes were found within the virgin timber, whereas all of the true second growth measured was of those age classes. At any rate, the elimination of this factor will tend not so much to reduce the rotation as to increase the yield.

The third factor is the density of present stands. Within the next century thinning will undoubtedly come into effect and will tend to increase the yield. Still another element which may be very important is the fact that many stands which are now pure yellow pine will in the future be a mixture of pine and white fir, with probably higher yields per acre than from the pure pine. Here again it is very difficult to forecast just how much additional yield per acre a change in composition will make, but whatever the change, yields will undoubtedly be increased and the use of the present table will therefore give conservative estimates of the productive capacity of the entire area.

The application of the data in Table II to the stands obtained after cutting is a distinct problem which this study has not attempted to solve. The yields obtained on fully stocked small plots can not be expected over large areas, and reductions will be necessary in employing the table.

YIELD OF WHITE FIR STANDS

Although the data do not permit any positive conclusions regarding the yields of white fir stands under different conditions of age and site, the indications are (fig. 11) that on a given area of land white

fir attains a greater average mature height than yellow pine; and that consequently land is site 3 for white fir which for yellow pine is only site 4. This tentative conclusion further indicates that more wood per acre can be produced on a given area by white fir than by yellow pine.

The indication is, indeed, that for stands with the same average mature heights, white fir produces more wood per acre than yellow pine. This can readily be explained by the greater ability of white fir to endure shade, and consequently to tend toward a greater num-

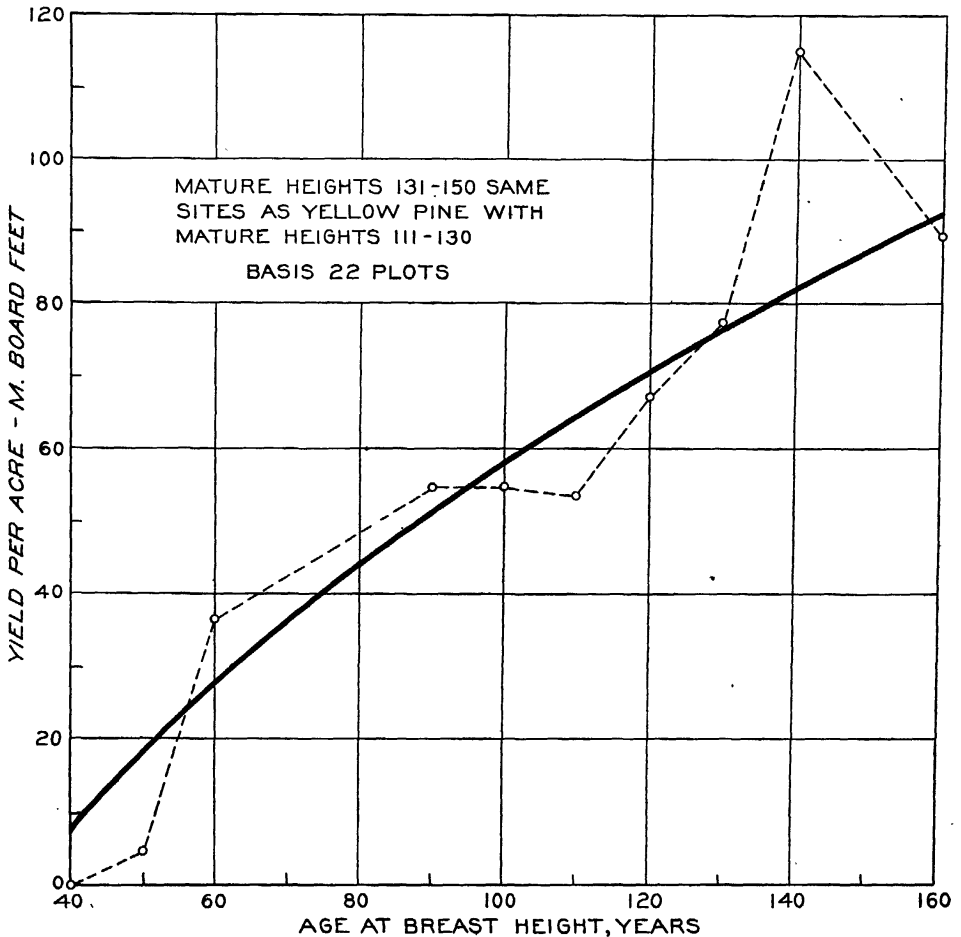


FIG. 11.—Yields per acre in white fir type

ber of trees per acre than yellow pine. It is thus probably true that white fir has decidedly the greater wood-producing capacity on a given piece of land. Whether future forest management shall favor pine or fir will depend primarily on the relative advantages of a high yield of less intrinsically valuable white fir and a lower yield of more valuable yellow pine.

SUMMARY

The present study indicates that on an average western yellow pine site, by preserving the young growth already on the ground under many of the virgin stands, a yield up to 40,000 board feet an acre can be obtained. This rate of growth is exceeded in several of

the western forest regions, but is probably higher than the yield in most of the subdivisions of the western yellow pine region. A rotation of about 120 years is indicated. However desirable shorter rotations may be, it does not appear that they can be realized and at the same time obtain the largest possible annual growth.

In the technique of the study several departures have been made from conventional practice. Of these perhaps the most important are (1) the use of dominant mature heights in the virgin forest as indices of site, (2) measurement of only a few heights on each plot, and construction of height-diameter curves for each as a basis for volume computations; (3) determination of ages by means of the increment borer; (4) employment of a log rule giving the actual merchantable contents of logs; and (5) adoption of standards of utilization not now in effect, but to be anticipated as effective when stands now young are utilized.

PRELIMINARY OBSERVATIONS ON AN INSECT OF THE COTTON STAINER GROUP NEW TO THE UNITED STATES¹

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INTRODUCTION

During the past year *Dysdercus obscuratus* Distant,² an insect of the cotton stainer group not hitherto recorded in the United States, has been found in a number of cotton fields in the lower Rio Grande Valley of Texas, as well as in cotton fields in Tamaulipas, Mexico, on the opposite side of the Rio Grande. Although no injury of note has as yet been observed which can be attributed to the attacks of this insect, several closely related species have long been recognized as very serious cotton pests in different parts of the world. It was therefore considered advisable to obtain as much preliminary information as possible concerning the life history and habits of this species.

OBSERVATIONS ON DYSDERCUS OBSCURATUS AT BROWNSVILLE, TEX.

The first specimens of this species collected in the United States were found by the writer near Brownsville, Tex., in July, 1922. They were fairly numerous, and a note made at the time of collection stated that they "were common on grass and weeds near Brownsville." Although some were seen in cotton fields, they could be found more easily in a number of places quite distant from cotton, and the writer gained the impression that the species was mainly a nectar feeder. In June, 1923, H. C. Millender and F. F. Bibby, field inspectors for the Federal Horticultural Board, collected numerous specimens of the insect in cotton fields in the vicinity of Matamoros, Mexico. These collections first directed attention to the possible economic importance of the insect.

PRESENT KNOWN RANGE OF THE SPECIES IN AMERICA

From information furnished by W. L. McAtee, this insect has previously been collected from Central America and Mexico, in the following localities: San Geronimo, San Juan, Guatemala; Cache, Costa Rica; Atoyac in Vera Cruz, Teapa in Tabasco, Mexico. In addition, T. E. Holloway and the writer collected specimens at Victoria, Tamaulipas, Mexico, in March, 1922, and the writer has recently (November, 1923) collected specimens from Tampico, Tamaulipas.

It may be remarked that specimens of three additional species of the cotton stainer genus have been collected by the writer in the Brownsville section, namely, *Dysdercus concinnus* Stal, *D. mimus*

¹ Received for publication Apr. 27, 1925; issued December, 1925.

² Determined by W. L. McAtee, of the Bureau of Biological Survey, U. S. Department of Agriculture.

Say, and *D. obliquus* H.-S., according to determinations made by McAtee. None of these species, however, has appeared in cotton fields.

DISTRIBUTION IN THE RIO GRANDE VALLEY

The species is known to occur over an area extending from the Gulf westerly to Donna, Tex., or a little more than 60 miles, and from the Mexican border north to Lyford, Tex., a distance of about 45 miles. The known area of distribution therefore contains several hundred square miles, and it is probable that this area will be considerably extended by careful scouting.

In general, it can be seen that the insect occurs, perhaps continuously, from Central America, and probably farther south, along the Gulf Coast of Mexico to the lower Rio Grande Valley of Texas.

MANNER OF SPREAD

Little is known regarding the manner in which the insect occupies new territory. The present indications are that it has been present in the Rio Grande Valley for an indefinite period, although not collected until recently. The adults are fairly strong fliers, and probably the area of infestation is extended mainly by flight. The young stages are gregarious and move in groups from plant to plant, but their progress is slow and extension in this way is relatively unimportant.

FOOD PLANTS

At Brownsville, Tex., the species has been found feeding in numbers upon at least four wild plants. These include (1) the plant which is apparently its natural host, *Sida carpinifolia* L., a member of the mallow family; (2) the common ragweed, *Ambrosia artemisiaefolia*; (3) wormwood, *Ambrosia elatior* L.; and (4) one of the sunflower family, *Verbesina encelioides* (Cav.) B. and H. Close observation of all these hosts has failed to reveal marked injury to flowers or seeds, and it appears that the feeding is mainly confined to sucking up the nectar produced by the plants. The writer has never been able, in the case of any plant, to find a definite injury to the living tissue which could be attributed to the attacks of the insects.

In addition, adults have been observed in considerable numbers in many localities upon cotton plants. At Donna and Mercedes, Tex., inspectors of the Federal Horticultural Board report having observed considerable numbers of the immature stages in the cotton fields, but always in decayed bolls. As will be shown later, the very young stages subsist chiefly upon decaying vegetable material, or, perhaps more correctly, upon the juices which they can extract from this material. Adults have been observed in small numbers within the blossoms of many different plants, evidently in search of nectar.

POSSIBLE ECONOMIC IMPORTANCE

Whether the insect is economically important has not yet been definitely determined, although numbers have been observed and collected in cotton fields. The most valuable field observation yet made was contributed by T. C. Richardson, of Lyford, Tex., for

several years agent of the Department of Agriculture for the county of Cameron, Tex. Mr. Richardson reported that during the cotton-growing season of 1923 a machine which he was using in his cotton fields for catching boll weevils picked up more of these cotton stainers than any other kind of insect. At the same time he harvested a crop of 22 bales of cotton from 25 acres of land and no perceptible staining of the lint was observed. This would indicate that no particular damage was inflicted upon the cotton, despite the numbers of the insect present, and it also tends to confirm the theory that the species feeds mainly upon the secretions of the nectary glands.

Although immature stages of the insect have been observed feeding within decaying cotton bolls on the plants, the adults have never been seen attacking a living boll, and no indications of injury have been observed upon the plants. The adults evidently rove at will, and gather in considerable numbers upon many different plants, especially upon those which, like the cotton plant, flower continuously. These insects seem to be essentially nectar-feeding. However, the fact should not be lost sight of that there are several closely related species which are important enemies of cotton.

The cotton-growing industry of the Rio Grande Valley is increasing rapidly, thousands of additional acres, mostly on freshly cleared land, being planted annually. The area maintaining the native host plants of the stainer is therefore being continually reduced. This is probably one of the main reasons for the sudden appearance of the insect in the cotton fields, vast numbers being forced to seek new food plants and new breeding territory. It is distinctly possible, therefore, that as this process is continued other host plants may be attacked. Since the genus in general has shown a marked preference for plants of the order Malvaceae, and since the natural host plants are being so largely displaced by the wholesale planting of cotton, it is reasonable to assume that the insects will be gradually driven into the cotton fields.

POSSIBILITY OF FUTURE SPREAD

With the information now available, the future advance of this insect can be only conjectural. The species has not yet been found beyond the limits of its main host plant, *Sida carpinifolia*. According to present information, its occurrence upon cotton and other plants of economic importance can be considered as more or less incidental to the wandering of the individuals in search of food. Unless the species should definitely transfer its breeding habits to other plants of economic importance, it now appears probable that its distribution will be mainly coincidental with the territory occupied by its principal host plant, but no definite prediction can be made.

STUDIES OF THE LIFE HISTORY OF THE INSECT

At the outset of work on the life history of the insect it was found difficult to rear the nymphs through the adult stage. After seven types of rearing cages had been tested and conditions gradually adapted to the requirements of the insects, much better results were obtained. Mating cages can now be stocked from the supply of insectary-reared adults. This could not be done when it was necessary to rely for breeding material on adults collected in the field.

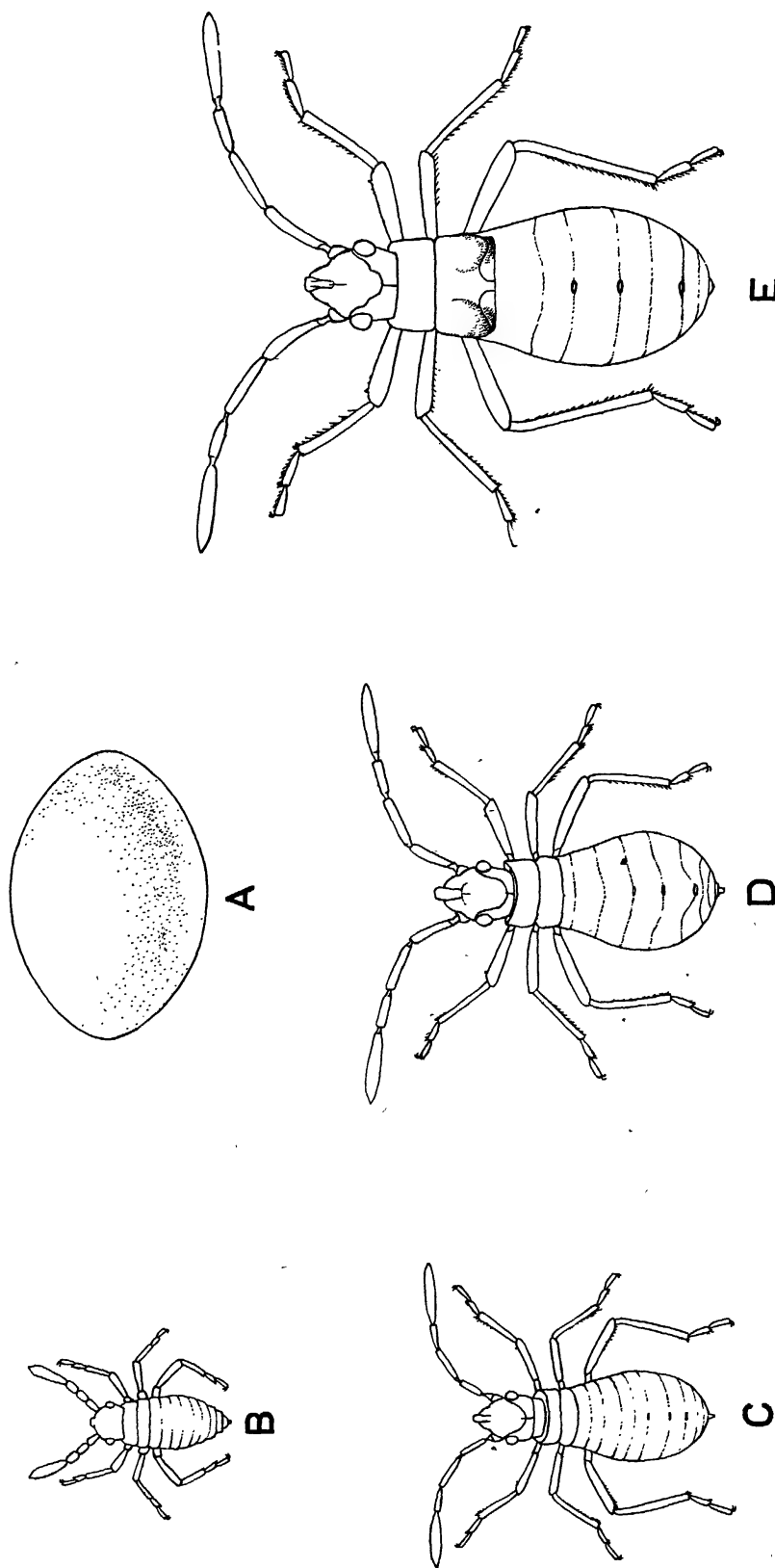


FIG. 1.—*Dysdercus obscuratus*: A, egg (25 times natural size); B, first-stage nymph; C, second-stage nymph; D, third-stage nymph; E, fourth-stage nymph (all drawings of nymphs 9 times natural size)

The type of rearing cage finally adopted consisted of large lantern globes, covered with cheesecloth on top, and with their bases inserted about 1 inch in soil in large trays. This soil was kept constantly moist. The host plants provided were planted in the soil and additional fruiting capsules were introduced into the cages when required for oviposition. This type of cage therefore provided a growing host plant, plenty of moist soil, and good ventilation through the cheesecloth cover. Moreover, in searching for eggs, the area of search was confined to the space covered by the base of the globe, or a circle about 4 inches in diameter, greatly simplifying the task of finding them.

All rearing was done on *Sida carpinifolia*, the native host plant of the insect, but present plans include a series of rearing tests on cotton.

NUMBER OF STAGES

It has been found that there are seven stages in the development of the insect. These include the egg, five nymphal stages, and the adult stage.

THE EGG

The eggs look very much like microscopic hens' eggs (fig. 1, A), being of about the same shape and similar in general proportions. They are about one twenty-fifth of an inch in length, and their greatest width, which is not exactly at the center of the egg, but slightly toward one end, is about two-thirds the length. When first deposited, the eggs are shiny, pearly-white, and semitransparent. As the incubation progresses they gradually turn a faint pink, and at the time of hatching are a light pinkish brown.

DURATION OF THE EGG STAGE

Up to the present 26 more or less exact incubation records have been obtained in the cages. It is difficult to make the incubation records with perfect accuracy owing to the female's habit of placing the egg under the surface of the ground, which sometimes results in the egg being overlooked until the second day. This probably occurred in two cases of the 26 considered, since one record of three days and one of four appeared too short in comparison with the average incubation period observed.

The 26 incubation records gave an average incubation period of 7.85 days. It should be taken into consideration, however, that life-history observations were not begun until after midsummer, and that a large proportion of these records were not secured until after cooler weather had arrived. In July and August, 5 records of 5 days each were obtained, as well as the two shorter records mentioned above. In September and October, 6 records of 6 days each were obtained, as well as 4 of 7 days, and several records of longer periods. On the other hand, in November and December the shortest incubation period recorded was 12 days, and the longest 17 days. Evidently the incubation period is about 5 days in midsummer and lengthens very considerably as the weather becomes cooler.

FIRST-STAGE NYMPH

The nymphs of the first stage are of about the same color as the nearly hatched eggs, a very light brown, with a pinkish tinge. They are a little larger than the eggs and slightly more elongate, and the legs and antennae are threadlike and colorless (fig. 1, B). It may be remarked that the head of the embryo nymph develops within the long-pointed end of the egg, and that the general contour of the body after hatching much resembles the shape of the egg. This stage is spent entirely underground, the nymph evidently feeding upon the supply of decaying food usually provided by the adult female at the time of oviposition.

DURATION OF THE FIRST STAGE

Twenty-one records of the duration of the first stage have been secured in the insectary. These cover a total of 132 days and give an average period of 6.29 days for this stage. As was the case with the incubation records, however, as well as with all subsequent records to be considered, cool weather undoubtedly resulted in the prolongation of many of them, so that the average secured is probably rather longer than the correct annual average. Since 8 records of 3 days were secured during the warm months of August, September, and early October, as well as 4 additional records of 4 days each, it seems evident that the midsummer period of the first stage is about 3 days. The longest record obtained was 19 days, from December 10 to December 29, 1923, but only 3 additional records were secured, each of 10 days or more.

SECOND-STAGE NYMPH

The nymphs of the second stage are about twice the size of those of the first stage. After the first molt the light color of the first stage is lost, and the nymphs of the second stage emerge bright red in color, slightly paler on the abdominal surface. The general shape of the insect can now be plainly distinguished, since it has attained a length of about one-eighth of an inch (fig. 1, C). The legs and antennae are now much longer and stronger, and in this stage the nymph is very active, being able to run about rapidly. Second-stage nymphs are sometimes, though rarely, seen above the surface of the ground, but usually, as in the case of the first stage, this stage is spent below the surface.

DURATION OF THE SECOND STAGE

Thirteen records of the second-stage nymphs have been secured, occupying a total of 109 days, thus giving an average period of 8.39 days. Four days are evidently the average midsummer duration of this stage, since four records of this length were secured in the warmer weather. The longest record covered 18 days, from December 11 to December 29, 1923.

THIRD-STAGE NYMPH

In the third stage the insect is about twice as large as in the second stage, and resembles it closely except in size (fig. 1, D). The color is still an even, bright red, but the head, thorax, and abdomen can now be seen to be divided by very faint whitish lines. This stage

is also very active and is usually the smallest stage to be found upon the plants under field conditions. The nymphs of the third stage, as well as the few nymphs of the second stage which have been observed above the ground, have a habit of dropping to the ground when the plant is disturbed and quickly hiding themselves in crevices in the soil. For this reason it is very difficult to collect them in numbers.

DURATION OF THE THIRD STAGE

Nine records of the third stage have been obtained, the period varying from 4 to 18 days. The total time covered by the nine records was 85 days, giving an average period of 9.44 days.

FOURTH-STAGE NYMPH

The nymph of the fourth stage is decidedly larger than that of the third, the insect now having attained a length of slightly more than one-fourth of an inch, exclusive of the antennae, which are about half as long as the body (fig. 1, E). Although the general color of the body is still bright red, the segmentation is now distinctly marked by delicate white lines on the dorsal surface. The abdominal surface is much paler than the dorsal, and the segments are joined by bright red lines. The wing pads now appear for the first time as two heavy black spots at the rear end of the thorax, and the legs and antennae are almost black. The nymphs of this stage are rather numerous in the field and can be collected without difficulty, since they are not nearly so shy as those of the third stage.

DURATION OF THE FOURTH STAGE

Eleven records of the fourth stage cover a total of 110 days, and give a general average period of 10 days. These periods range from 4 to 19 days. Evidently the midsummer average period is about 4 or 5 days for this stage.

FIFTH-STAGE NYMPH

In the fifth stage the sexes differ considerably; so much so that at first they were thought to be two separate stages. The female nymph of the fifth stage is considerably larger than the male nymph of the same stage owing to the much larger abdomen, which makes the wing pads appear proportionately shorter. The fifth-stage male nymph resembles the adult much more closely than does the female, since the abdomen is not so large and the wing pads therefore lie flat along the back, appearing to be longer in proportion to the size of the abdomen (fig. 2, A).

The length of the body in the fifth stage averages about three-eighths of an inch, exclusive of the antennae. The wing pads are now about twice as large as they were in the fourth stage. The general coloration is much the same as before except that the segments of the body are very distinct, the dividing lines having become much more prominent.

DURATION OF THE FIFTH STAGE

All of the preceding records of the insect, from the incubation of the egg to the fourth-stage nymph, have been based upon mass observations, all the individuals present in one cage being utilized

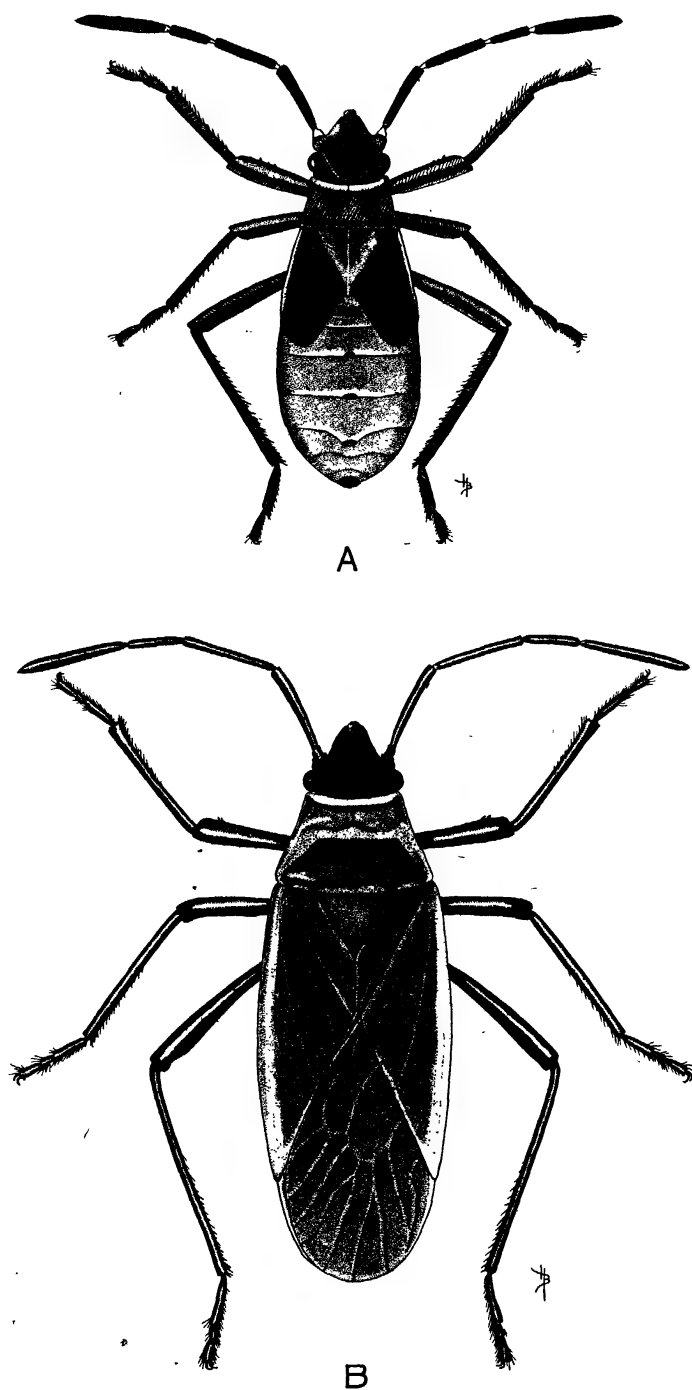


Fig. 2.—*Dysdercus obscuratus*. A, Fifth-stage nymph; B, adult. $\times 6$

to make one record. A single record is therefore based upon the development of possibly a considerable number of individuals. In the case of the fifth-stage nymph, however, the number of specimens which were carried successfully through their entire life history, from

egg to adult, was only 31. These 31 records are therefore considered individually.

The total length of time occupied by these 31 fifth-stage records was 564 days, giving an individual average of 18.2 days each. The individual variation was from 10 to 29 days. Since, however, the first adult reared from the egg in the cages appeared on October 7, 1923, and all of the remainder were reared during the months of October, November, and December, 1923, it is evident that their life-history periods were longer than they would have been during the hotter months of midsummer.

TOTAL PERIOD OF DEVELOPMENT

From the data presented it is apparent that great variation exists between the total length of time required for development during the months of midsummer and those of fall and winter when the periods of development are considerably extended because of lower temperatures. Table I shows this more clearly, and presents the information in a more concise form.

TABLE I.—*Developmental periods of Dysdercus obscuratus at different seasons*

Stage	Midsummer period	Fall period	Winter period	Total number of records	Total number of days	Average period of develop- ment
	<i>Days</i>	<i>Days</i>	<i>Days</i>			<i>Days</i>
Incubation.....	5	8	12 to 17	26	-----	7.85
First stage.....	3	5	10 to 13	21	132	6.29
Second stage.....	4	6	11 to 13	13	109	8.39
Third stage.....	5	9	15 to 18	9	85	9.44
Fourth stage.....	5	7	15 to 19	11	110	10.06
Fifth stage.....	10	20	24 to 28	31	564	18.20
Total development.....	32	55	87 to 108	111	1,000	60.17

THE ADULT

The adults are brightly colored, slightly more than one-half inch in length, the antennae adding to this about three-eighths of an inch (fig. 2, B). The head is coral red, with a narrow white band between the head and thorax. The thorax contains a transverse band of coral red, followed by a narrow yellow band, with a transverse black ridge at the base of the wings. The predominating colors of the wings are black and yellow, with faint white margins. The coloration of the wings varies considerably, irrespective of sex, some adults having much more yellow than black, others having nearly black wings with little yellow showing. The legs and antennæ are orange at the base, with black extremities. The abdomen is bright red dorsally, with white lines between the segments. Ventrally the abdomen is white with a bluish tinge, and has bright red lines between the segments.

It is rather difficult to distinguish the sexes from each other. Usually the female is slightly larger than the male, and the abdomen is larger and stouter. The most easily distinguished sexual character is the wider red bands across the ventral surface of the abdomen of the male, particularly the last three segments.

LONGEVITY OF THE ADULT

Only eight complete longevity records have been secured from observations in the mating cages, four of these being for females and four for males. The four females lived a total of 199 days, giving an average female longevity of 49.8 days. The four males lived a total of 193 days, giving an average male longevity of 48.25 days. The maximum individual longevity of the females was 55 days; the maximum male longevity 73 days. Although little difference between the longevity of the sexes is shown above, there are indications that on an average the females are longer-lived than the males. The survival of 37 field-collected females in the mating cages gave an average survival of 10.92 days from the date of collection. Thirty-six males lived an average of 9 days. Of course no data were available showing the ages of these adults at the time of collection.

HABITS OF THE SPECIES

The adults are gregarious, not only in regard to each other but also in regard to the larger stages of the immature forms. They are commonly found in groups on the plants from which they derive their nourishment, the groups including both sexes of the adult and frequently considerable numbers of nymphs of the third, fourth, and fifth stages; sometimes, but very rarely, nymphs of the second stage. These groups move about from plant to plant, keeping more or less together, the extent of their movements apparently being controlled by the quantity of food which they are able to find. Frequently great numbers of the insects concentrate upon a certain group of plants which are evidently supplied with large quantities of the sought-for juices, and possibly hundreds of the insects will be found upon the plants for several days, after which, having apparently exhausted the food supply, they migrate to other plants in the neighborhood. The plants which have been "worked over" do not appear to be damaged to any appreciable extent by the wholesale visitation, although stalks have several times been observed so weighted with the insects that they have been bent nearly to the ground.

Mating appears to occur at any time after the adults have reached the age of 2 days. The eggs are generally deposited under the surface of the ground, a dead leaf or fruiting capsule of the host plant usually being buried with them for food for the nymphs after hatching. When no leaf or fallen capsule is available, the females sometimes oviposit in the soil immediately around the stem of the host plant where it emerges from the ground. Occasionally a simple groove in the ground is used for oviposition. Eggs may occur singly or in clusters of from 2 to 25 or 30, adhering together very slightly, although easily separable without damage. The act of oviposition has been observed on two or three occasions. When a suitable capsule had been selected, the female would deposit two or three eggs, then carefully cover them with soil, pulling the particles over them with her feet. More eggs would then be deposited and covered in their turn, until finally the entire capsule would be buried under the surface. These observations were all made under insectary conditions, since

the closest search has as yet failed to disclose eggs in the field under natural conditions.

From the manner of oviposition, it is very difficult to determine the exact number of eggs deposited. Although a considerable number of eggs were secured in cages containing no soil, there did not appear to be as many per female as there should have been, and it is probable that oviposition was curtailed by unnatural conditions. The greatest number of eggs definitely counted from two females was 107, laid on two consecutive nights (56 eggs on the night of July 26-27 and 51 eggs on the night of July 27-28). No eggs were subsequently deposited, so that the eggs, in this case, averaged 53.5 per female. Usually, however, only about 15 to 30 eggs were obtained from each female in this type of cage. It has not been possible to make definite counts of eggs deposited underground in the cages containing soil.

SUMMARY

During the past year *Dysdercus obscuratus* Distant, an insect of the cotton stainer group new to the United States, has been found in cotton fields in Texas.

The insect occurs, perhaps continuously, from Central America, and probably farther south, along the Gulf Coast of Mexico to the lower Rio Grande Valley of Texas.

Probably the area of infestation is extended mainly by flight.

At Brownsville, Tex., the species feeds upon four wild plants, *Sida carpinifolia* L., the common ragweed (*Ambrosia artemisiifolia*), wormwood (*Ambrosia elatior* L.), and *Verbesina encelioides* (Cav.) B. and H.

Whether the insect is economically important has not been definitely determined.

The eggs are deposited in clusters, usually under the surface of the ground. The incubation period is about five days in midsummer.

The nymph has five stages. The duration of each stage varies with the time of year in which it occurs.

In the lower Rio Grande Valley the average period of development from egg deposition to the appearance of the adult insect was found to average 32 days in midsummer, 55 days in the fall, and to range from 87 to 108 days in the winter.

Male adults in cages were found to live an average of 48.25 days; females in cages lived an average of 49.8 days.

The adults and larger instars of the nymph stage are gregarious, being found commonly in groups on the plants from which they derive nourishment. These groups move about from plant to plant. Frequently great numbers of insects will concentrate upon a certain group of plants and remain there for several days.

NITROGEN METABOLISM IN ETIOLATED CORN
SEEDLINGS¹

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INTRODUCTION

The process of seed germination is of great significance as a means of attaining deeper and more extensive knowledge concerning the physiology of plants. The universal importance of cereals as food and feed has made it desirable to take up a systematic study of these plants with the object of throwing additional light on the fundamental physiological and biochemical processes involved in the germination of various cereal seeds. Because of the vast field covered by the phenomena in question it was necessary to limit the scope of the investigation, and the work has therefore been confined chiefly to the study of the nitrogenous compounds (proteins and nonproteins) in cereals. Some interesting studies (14, 16) of these compounds in relation to the nutrition of man and animals have been made.

Before the germination work proper was taken up, preliminary experiments showed that wheat (*Triticum vulgare*) (9),² oats (*Avena sativa*) (10), corn (*Zea mays*) (11), and rye (*Secale cereale*) (12) contain polypeptides and free amino acids in their ungerminated kernels.

There is a great deal of information at hand concerning the process of germination. In some seedlings the proportions of acid amides and amino acids are fairly uniform at certain stages of germination; at other stages the acid amides prevail. Some seedlings, like those of grasses and legumes, are rich in asparagine; the seedlings of others, like spinach and black radish, are rich in glutamine; and the seedlings of still others, like the sunflower and pumpkin, contain both asparagine and glutamine, though one of them ordinarily in predominant proportion (18). As to the interrelationship and significance of these facts we do not know much beyond the hypothesis of Schulze, that during germination the amino acids are gradually converted into asparagine or glutamine (17).

So far as the occurrence of polypeptides in seedlings is concerned, we have no information whatever, nor do we have sufficient knowledge with regard to the humin bodies met with in seedlings.

It is well known that plant proteins may be split by chemical means (acids, alkalies), by bacteria, and by enzymes naturally occur-

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² Reference is made by number (*italic*) to "Literature cited," p. 1163.

ring in plant tissues. Whereas hydrolysis of plant proteins with strong acids leads to amino acids, bacterial action when long enough continued leads to secondary products (fatty acids, amines) through deamination and decarboxylation. On the other hand, proteolysis under the influence of enzymes ordinarily present in seeds, seedlings, and generally in various plant tissues yields not only amino acids, but also asparagine and glutamine, instead of aspartic and glutaminic acids, which are obtained by acid hydrolysis. Whether asparagine and glutamine are formed secondarily from amino acids as advanced by Schulze (17) needs further confirmation. It is, therefore, not surprising that studies on the physiological processes which take place during seed germination, involving as they do catabolism of the nitrogenous seed compounds on the one hand and anabolism of new nitrogen compounds in the growing parts of the seedling on the other are very far from being complete. Nearly every investigator emphasizes the necessity of further studies of the physiological processes in question. Thus Brown and Morris (2) state:

The complex metabolic processes which take place during the germination of seeds have attracted, from the time of Th. De Saussure, a large amount of attention * * * nevertheless at the present time we are far from possessing an exact knowledge. * * *

Toole (21), says:

Although the volume of literature on seed germination is large, knowledge of the transformations which occur in the seed and seedling during germination is far from complete. Also, the data on the influence of specific external factors on germination are confusing. * * *

In the study of seed germination herein reported etiolated corn seedlings only have been used. There is probably, during the first stage of germination, no essential difference between seedlings grown in the presence of light and those grown in its absence. As soon, however, as chlorophyll develops in the seedlings there is, along with the degradation of the seed proteins, which are so well known through the excellent work of Osborne (13, 14, 15) Chittenden (3), and their collaborators, considerable accumulation of carbohydrates in the seedlings by photosynthesis, which renders the estimation and isolation of the various nitrogenous disintegration products very difficult, and hence not quite accurate. On the other hand, etiolated seedlings ordinarily use up the seed carbohydrates from which they derive the continual supply of energy necessary for vital activities. The nitrogen compounds in such seedlings, therefore, become gradually more concentrated.

METHODS

In order to obtain comparable results, the writer tried to maintain as uniform conditions as possible throughout this work. Thus, he used for his experiments the same variety of corn (Four County corn), which was bred by the Four County Grain Improvement Association at Ackley, Iowa, under the direction of the Iowa Agricultural Experiment Station. The seed was planted on perforated copper trays fairly uniformly, about 400 kernels per tray, between moist absorbent paper towels, and kept in a dark room at the constant temperature of 25° C. for the desired period. Only occasionally the temperature would rise to 26° or 27°. No watering whatever

was done, the moisture within the germinator being maintained by a pan filled with water and placed at the bottom of the germinator. A small portion of the seed, to be mentioned subsequently, was planted in pots filled with sand which was thoroughly washed and contained but a negligible quantity of nitrogen. This seed, too, was placed in a dark room at the temperature of 27° C.

Tests with three samples of seed planted in sand at the temperature of 27° C. gave 93, 93, and 95 per cent germination. Two other samples in which the seed was put between moist absorbent paper towels and kept at 30° C. gave 95 and 90 per cent germination, thus proving the high quality of the seed.

One difficulty encountered in the work was the molding of the seedlings. Whereas seedlings which were allowed to grow from two to four days were practically free from mold, those which grew for a longer period were more or less covered by a growth of fungi, among which *Rhizopus nigricans* was conspicuous. In an attempt to do away with the fungi the corn seed was treated with a 0.25 per cent solution of uspulun (a chlorophenol mercury compound) for 30 minutes, at the expiration of which the seed was washed with water and immediately planted with the aid of an uspulun-treated spatula. This treatment, although helpful to a certain extent, did not obviate the difficulties entirely. Nor could the appearance of fungi be prevented by spraying with mercuric chloride solution (1 : 1,000). Whether untreated or uspulun-treated seed was allowed to germinate, in each case only material free from mold was taken for work. The seedlings, including the seed, were dried in an electric drying oven at 60° C. for from two to three days, after which they were ground in a mill until all had passed through a 40-mesh sieve, and put into jars ready for use.

The total nitrogen was estimated by the Gunning modification of the Kjeldahl method.

The protein nitrogen was determined according to Stutzer's method as outlined in previous publications (6, 7, 8).

The nonprotein nitrogen was estimated by determining the nitrogen in the filtrate from the protein precipitate, as obtained by means of Stutzer's copper solution (20), or it was calculated by difference from a hundred.

The methods for estimation of acid amides, amino acids, polypeptides and other constituents will subsequently be described in this paper.

The results secured are recorded in the following tables.

EXPERIMENTAL DATA

An examination of Table I shows that the protein nitrogen fell rapidly with the progress of germination. Thus, from 95.68 per cent in the ungerminated seed, calculated on the basis of the total nitrogen, it fell to 77.63, 60.74, 61.95, and 57.36 per cent after 2, 4, 5, and 8 days, respectively, in the untreated seedlings with the paper substratum. Similarly, in the uspulun-treated seedlings the protein nitrogen decreased to 61.07, 54.52, and 55.26 per cent after 4, 5, and 8 days, respectively. That the protein nitrogen fell very rapidly from 95.68 per cent in the seed to 58.02 per cent in the seedlings which were grown in sand for but 3 days may be attributed to the considerably higher moisture conditions of the sand seedlings.

TABLE I.—Proportion of total, of protein, and of nonprotein nitrogen in the various seedlings

No.	Conditions of germination				Total nitrogen on the basis of—		Protein nitrogen on the basis of—		Nonprotein nitrogen			
	Du- ra- tion	Substratum	Tem- pera- ture	Treatment	Oven- dried seed- lings	Orig- inal oven- dried seed	Oven- dried seed- lings	Total nitro- gen	Oven- dried seed- lings	Total nitro- gen	Oven- dried seed- lings	Total nitro- gen
	Days		° C.		P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1	3	Sand.....	27	None.....	1.75	-----	0.97	56.78	0.69	40.60	0.74	43.22
2	3	do.....	27	do.....	1.69	-----	1.01	59.26	-----	-----	.70	40.74
3	3	do.....	27	do.....	1.69	-----	-----	-----	-----	-----	-----	-----
		Average.....	-----	-----	1.71	-----	.99	58.02	.69	40.60	.72	41.98
4	4	Sand.....	27	None.....	1.86	-----	-----	-----	-----	-----	-----	-----
5	4	do.....	27	do.....	1.90	-----	-----	-----	-----	-----	-----	-----
		Average.....	-----	-----	1.88	-----	-----	-----	-----	-----	-----	-----
6	2	Paper.....	30	None.....	1.77	1.71	1.36	79.33	.37	21.53	.36	20.67
7	2	do.....	30	do.....	1.71	1.65	1.31	75.93	.49	28.33	.41	24.07
8	2	do.....	30	do.....	1.69	1.63	-----	-----	-----	-----	-----	-----
		Average.....	-----	-----	1.72	1.66	1.33	77.63	.43	24.93	.38	22.37
9	4	Paper.....	30	None.....	1.76	1.63	1.08	58.80	-----	-----	.75	41.20
10	4	do.....	30	do.....	1.89	1.74	1.15	62.68	.72	39.63	.68	37.32
11	4	do.....	30	do.....	1.87	1.73	-----	-----	-----	-----	-----	-----
12	4	do.....	30	do.....	1.79	1.65	-----	-----	-----	-----	-----	-----
		Average.....	-----	-----	1.83	1.69	1.12	60.74	.72	39.63	.72	39.26
13	5	Paper.....	25	None.....	1.80	1.61	1.11	61.45	.69	38.20	.69	38.55
14	5	do.....	25	do.....	1.80	1.61	1.12	62.45	.67	37.10	.68	37.55
		Average.....	-----	-----	1.80	1.61	1.12	61.95	.68	37.65	.69	38.05
15	8	Paper.....	30	None.....	2.10	1.73	1.16	55.38	1.01	48.09	.93	44.62
16	8	do.....	30	do.....	2.11	1.74	1.19	57.06	.92	44.23	.90	42.94
17	8	do.....	30	do.....	2.06	1.70	1.24	59.12	.92	44.16	.85	40.88
		do.....	30	do.....	2.06	1.70	1.21	57.88	.93	44.37	.88	42.12
		Average.....	-----	-----	2.09	1.72	1.20	57.36	.95	45.21	.89	42.64
18	4	Paper.....	25	Uspulun..	1.78	1.65	1.13	63.34	.65	36.50	.67	36.66
19	4	do.....	25	do.....	1.78	1.65	1.05	58.80	.73	41.00	.75	41.20
		Average.....	-----	-----	1.78	1.65	1.09	61.07	.69	38.75	.71	38.93
20	5	Paper.....	25	Uspulun..	1.85	1.66	.96	52.11	.88	48.01	.88	47.89
21	5	do.....	25	do.....	1.83	1.65	1.05	56.92	-----	-----	.79	43.08
		Average.....	-----	-----	1.84	1.66	1.01	54.52	.88	48.01	.84	45.48
22	8	Paper.....	25	Uspulun..	1.83	1.54	1.02	56.24	.82	44.93	.80	43.76
23	8	do.....	25	do.....	1.81	1.53	.99	54.27	.89	49.11	.83	45.73
		Average.....	-----	-----	1.82	1.54	1.01	55.26	.86	47.02	.82	44.74
24	11	Paper.....	25	Uspulun..	1.96	-----	1.06	55.28	.82	42.92	.85	44.72
25	11	do.....	25	do.....	1.86	-----	1.12	58.78	-----	-----	.79	41.22
		Average.....	-----	-----	1.91	-----	1.09	57.03	.82	42.92	.82	42.97
26	15	Paper.....	25	Uspulun..	1.93	-----	1.04	56.14	.82	44.10	.81	43.86
27	15	do.....	25	do.....	1.77	-----	1.04	55.98	.82	44.18	.81	44.02
		Average.....	-----	-----	1.85	-----	1.04	56.06	.82	44.14	.81	43.94
28	-----	Ungerminated seed..	-----	-----	1.73	-----	1.64	95.72	.07	4.21	.07	4.28
29	-----	do.....	-----	-----	1.71	-----	-----	95.64	.08	4.41	.07	4.36
		Average.....	-----	-----	1.72	-----	1.64	95.68	.08	4.31	.07	4.32

Beginning with the eighth day and continuing to the fifteenth there was a slight increase in the protein nitrogen. There would seem to be an effort on the part of the etiolated seedlings to synthesize proteins. For this process they needed energy, which, in the absence of light, they derived from breaking down the reserve materials of the seed, such as the starch.

The figures for the nonprotein nitrogen, found by direct estimation, quite naturally stand in reverse ratio to the protein nitrogen. Thus, the ungerminated seed (Nos. 28 and 29) with the highest protein nitrogen (95.68 per cent) has the lowest nonprotein nitrogen (4.31 per cent). Equally, the five-day uspulun-treated seedlings (Nos. 20 and 21) with the lowest protein nitrogen (54.52 per cent) have the highest nonprotein nitrogen (48.01 per cent).

The percentage of total nitrogen, it will be noticed, increases somewhat with the duration of germination, when calculated on the basis of the oven-dried seedlings. In this connection it should be remembered that the whole of the seedlings, including the seed, was taken into the work. Therefore, strictly speaking, the total nitrogen of the seedlings should be equal to the total nitrogen of the seed, provided the weight of the seedlings (including the unutilized seed) at any time would be equal to that of the ungerminated seed. Actually, however, the weight of the former decreases more and more as germination progresses, because the seedlings, in the absence of light, derive the energy necessary for growth, respiration, and other vital activities from oxidation or cleavage of the stored-up materials. If only non-nitrogenous compounds such as starch were utilized for the creation of energy, then the total nitrogen calculated on the basis of the oven-dried seedlings should, with the progress of germination, gradually become greater than the nitrogen proportion in the ungerminated seed. However, the growing seedlings can and do obtain the necessary energy also by the cleavage and further degradation of nitrogenous compounds such as proteins, as is evident from the observation on proteolytic enzymes reported subsequently. Since this decomposition may be accompanied by loss of nitrogen, it has the tendency to decrease the proportion of nitrogen in the seedlings. It is for these reasons that the total nitrogen was calculated to the oven-dried seedlings as well as to the seed. The circumstance that the total nitrogen calculated on the seedlings is higher than in the ungerminated seed is an indication that the growing seedlings derive the necessary energy chiefly at the expense of the nonnitrogenous compounds, such as starch. This is also corroborated by the observation that the aqueous extracts of the seedlings contained less starch the further the germination progressed.

In order to recalculate the nitrogen found directly in the seedlings to the oven-dried seed, experiments were made to ascertain the change of weight of the seed on germination. Each of several perforated copper trays was covered with absorbent paper towels and divided into equal parallel sections. On each of these there were planted 10 uniform-looking corn kernels, the weight of which was accurately determined. On covering the seed with absorbent paper, the whole was thoroughly sprinkled with water and allowed to germinate in a dark room under exactly the same conditions as described above. After the expiration of 2, 4, and more days, all of the seed-

lings of the clean-looking rows were weighed, then dried to constant weight at 105° C. and weighed again. The results are recorded in Table II.

TABLE II.—*Change of weight in the various seedlings during germination*

No.	Conditions of germination				Weight of ungerminated seed (10 kernels)		Weight of oven-dried seedlings	Ungerminated oven-dried seed
	Duration	Substratum	Temperature	Treatment	Air-dry	Oven-dried		
	Days		° C.		Grams	Grams	Grams	Per cent
1	2	Paper.....	25	None.....	3.8200	3.6080	3.4914	96.77
2	2	do.....	25	do.....	3.7408	3.5332	3.4088	96.48
		Average.....						96.63
3	4	Paper.....	25	None.....	3.7200	3.5135	3.2460	92.39
4	4	do.....	25	do.....	3.6784	3.4742	3.2034	92.21
		Average.....						92.30
5	5	Paper.....	25	None.....	3.9100	3.6930	3.2746	88.67
6	5	do.....	25	do.....	3.9637	3.7437	3.3631	89.83
		Average.....						89.25
7	8	Paper.....	25	None.....	3.6916	3.4867	2.8093	80.57
8	8	do.....	25	do.....	3.8727	3.6578	3.0856	84.36
		Average.....						82.47
9	2	Paper.....	25	Uspulun.....	3.9900	3.7686	3.6281	96.27
10	4	do.....	25	do.....	3.7891	3.5788	3.3104	92.50
11	5	do.....	25	do.....	3.4386	3.2478	2.9208	89.93
12	8	do.....	25	do.....	3.5213	3.3259	2.8015	84.23

A glance at Table II shows that after 2, 4, 5, and 8 days of germination the weight of the seed was reduced, respectively, to 96.63, 92.30, 89.25, and 82.47 per cent. Equally, in the case of the uspulun-treated seed, it decreased to 96.27, 92.50, 89.93, and 84.23 per cent, after 2, 4, 5, and 8 days respectively. By means of this table the recalculation of the total nitrogen to the oven-dried seed (column 7 of Table I) is very simple. Thus, for example, No. 6 (Table I, column 6) which, after 2 days' germination was found to contain 1.77 per cent of nitrogen, calculated on the basis of the oven-dried seedlings, has 1.77×0.9663 , or 1.71 per cent of nitrogen calculated on the oven-dried seed. The same considerations apply to the other tables.

AQUEOUS EXTRACTS OF THE SEEDLINGS

Although the results presented in Table I clearly establish the fact that the compounds constituting the nonprotein nitrogen are increasing with the progress of the germination, yet it seemed desirable to corroborate this finding by direct extraction with water, which, moreover, was necessary for the succeeding experiments. Accordingly, aqueous extracts were prepared as follows. Ten-gram portions of flour of the various seedlings were treated with 200 c. c. of boiling hot ammonia-free water and kept on a water bath for 30 minutes, after which the whole was centrifuged or filtered. The solid residues were treated once more in like manner. Both extracts were combined and made up to 500 c. c., of which two portions of

200 c. c. each were oxidized according to Kjeldahl's method; the remaining 100 c. c. were used for qualitative tests as well as for acidity estimation (first series). In order to obtain a more concentrated extract suitable for the trials reported in the subsequent pages, another series of extracts was prepared, in which 50-gram portions of flour were treated with hot ammonia-free water, kept on the water bath for 15 minutes, and then centrifuged. The solid residues were extracted two more times. The three extracts were made up to 500 c. c. in 25 c. c. portions, the nitrogen of which was determined according to the Kjeldahl method. The results in question are recorded in Table III.

TABLE III.—Percentage of nitrogen extracted by water from the various seedlings

No.	Conditions of germination				Nitrogen extracted (first series) on the basis of—			Nitrogen extracted (second series) on the basis of—		
	Duration	Substratum	Temperature	Treatment	Oven-dried seedlings	Original oven-dried seed	Total nitrogen of seedlings	Oven-dried seedlings	Original oven-dried seed	Total nitrogen of seedlings
	<i>Days</i>		<i>° C.</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	3	Sand.....	27	None.....	0.845	-----	49.39	-----	-----	-----
2	3	do.....	27	do.....	.834	-----	48.77	-----	-----	-----
		Average.....			.840	-----	49.08	-----	-----	-----
3	4	Sand.....	27	None.....	.990	-----	52.63	-----	-----	-----
4	4	do.....	27	do.....	.990	-----	52.51	-----	-----	-----
		Average.....			.990	-----	52.57	-----	-----	-----
5	2	Paper.....	30	None.....	.434	0.419	25.22	0.382	0.369	22.22
6	2	do.....	30	do.....	.432	.417	25.09	.370	.358	21.52
		Average.....			.433	.418	25.16	.376	.364	21.87
7	4	Paper.....	30	None.....	.808	.746	44.18	.722	.666	39.53
8	4	do.....	30	do.....	.808	.746	44.18	.762	.703	41.63
		Average.....			.808	.746	44.18	.742	.685	40.58
9	8	Paper.....	30	None.....	.998	.823	47.76	.964	.795	46.14
10	8	do.....	30	do.....	1.014	.836	48.50	.919	.758	43.96
		Average.....			1.006	.830	48.13	.942	.777	45.05
11	4	Paper.....	25	Uspulun.....	.695	.643	39.05	.749	.693	42.05
12	4	do.....	25	do.....	.696	.644	39.10	-----	-----	-----
		Average.....			.696	.644	39.08	.749	.693	42.05
13	8	Paper.....	25	Uspulun.....	.965	.813	53.01	.968	.815	53.18
14	8	do.....	25	do.....	.928	.782	51.00	.941	.793	51.71
		Average.....			.947	.798	52.01	.955	.804	52.45
15	11	Paper.....	25	Uspulun.....	.966	-----	50.56	.974	-----	50.98
16	11	do.....	25	do.....	1.000	-----	52.53	.955	-----	50.02
		Average.....			.983	-----	51.45	.965	-----	50.50

A glance at Table III shows at once that the percentage of nitrogen that could be extracted by water increases as the germination progresses. Thus, the seedlings which grew on sand at 27° for 3 days yielded an extract which contained 49.08 per cent of water-soluble nitrogen, calculated to their total nitrogen. This figure rose to 52.57

per cent in seedlings which grew for 4 days. In like manner seedlings which grew between moist paper towels for 2, 4, and 8 days yielded extracts which contained, respectively, 25.16, 44.18, and 48.13 per cent of nitrogen. Similar results were obtained with the seedlings whose seed was treated with uspulun solution. That the extracts of the second series have, as a rule, a lower nitrogen content than the corresponding extracts of the first series is due to the fact that in the latter series a considerably larger quantity of water was used for the extractions. The higher nitrogen content of the aqueous extracts in both series as compared with the nonprotein nitrogen in Table I was to be expected, because the water extracts contain, in addition to the nonprotein nitrogen compounds, also the nitrogen of soluble proteins such as albumin and proteose.

ACIDITY OF THE AQUEOUS EXTRACTS

In the course of the work it was noticed that the water extracts of the different seedlings showed a more or less pronounced acid reaction. It was deemed of considerable interest to find out whether or not there is a definite relationship between the acidity on the one side and the conditions of germination on the other. With this object in view the aqueous extracts used in Table III were applied to the acidity estimations. Inasmuch as the aqueous extracts of the various seedlings were more or less colored, the color intensity increasing with the length of the germination, it was thought best uniformly to dilute all of the extracts with water so as to have identical dilutions capable of giving sharp titration end points. Accordingly, the following procedure was employed: To 20 c. c. of the water extracts in question diluted with 100 c. c. of distilled water, which was neutral to phenolphthalein, 10 drops of this indicator were added and directly titrated with N/10 sodium hydroxide. The data obtained are recorded in Table IV.

In glancing over Table IV it will be seen that the extract of the ungerminated seed (Nos. 17 and 18) showed the lowest acidity, which could be neutralized with but 0.25 c. c. tenth-normal sodium hydroxide. The acidity rose in the extracts of the seedlings that grew on sand for three and four days (Nos. 1 to 4) to 0.91 and 1.26, respectively. In like manner the extracts of seedlings that were grown on paper for 2, 4, and 8 days (Nos. 5 to 10), respectively, show a gradually increasing acidity of 0.39, 0.73, and 1.19, respectively. Similarly, the extracts of seedlings, previously treated with uspulun solution, which were grown on paper for 4, 8, and 11 days (Nos. 11 to 16) showed the acidities of 0.71, 0.93, and 1.12, respectively. Thus, in all cases the acidity may be said to have risen with the duration of the germination. The differences in acidity between the ungerminated seed on the one hand and the seedlings on the other are so great as to make unnecessary the recalculation to the weight of the original oven-dried seed.

TABLE IV.—Acidity percentage of the various seedling extracts

[Twenty cubic centimeters of extract used in each case]

No.	Conditions of germination				Weight of oven-dried seedlings corresponding to extract used	N/10 NaOH used for neutralization	Average
	Duration	Substratum	Temperature	Treatment			
	Days		° C.		Grams	C. c.	C. c.
1	3	Sand	27	None	0.3706	0.91	0.91
2	3	do.	27	do.	.3706	.91	
3	4	do.	27	do.	.3647	1.24	1.26
4	4	do.	27	do.	.3647	1.28	
5	2	Paper	30	do.	.3737	.38	.39
6	2	do.	30	do.	.3737	.40	
7	4	do.	30	do.	.3726	.73	.73
8	4	do.	30	do.	.3726	.72	
9	8	do.	30	do.	.3752	1.18	1.19
10	8	do.	30	do.	.3752	1.20	
11	4	do.	25	Uspulum	.3764	.72	.71
12	4	do.	25		.3764	.71	
13	8	do.	25		.3764	.70	
14	8	do.	25	do.	.3756	.92	.93
15	11	do.	25	do.	.3756	.94	
16	11	do.	25	do.	.3773	1.07	1.12
17		Ungerminated seed		do.	.3773	1.16	
18		do.		do.	.3658	.24	.25
		do.		do.	.3658	.26	

Inasmuch as the observation was made by Schulze (19) and others that the organic phosphorous compounds present in seeds are gradually converted into inorganic phosphoric acid, as germination of the seeds progresses, the idea suggested itself that the acidity of the aqueous extracts of the seedlings might be due to this very reason. Although no quantitative analyses of the inorganic phosphoric acid occurring in the water extracts of the seedlings were run, repeated qualitative tests seemed to point to the fact that the rise in the acidity of the seedling extracts is to be attributed at least in part to the increase of the inorganic phosphoric acid.

DISTRIBUTION OF THE WATER-SOLUBLE NITROGEN IN THE SEEDLINGS

In order to establish the chemical nature of the organic nitrogenous compounds met with in the water extracts of the various seedlings, the following experiments were made. Ordinarily 100 or 50 gram portions of seedling flour were treated with boiling hot water and heated on the water bath for 15 minutes, after which the whole was centrifuged. The solid residues were treated twice more in like manner. The three extracts were now combined, refiltered if necessary, and made up to 1,000 c. c. or 500 c. c. In two portions of 25 c. c. each the nitrogen was estimated according to Kjeldahl's method, which gave the total water-soluble nitrogen (*a*).

From 400 c. c. of the remaining extract proteins were removed by slightly acidifying the extract with acetic acid and adding a 10 per

cent tannic acid solution as long as precipitation occurred, excess of the precipitant being avoided. After 24 hours, or sooner, the precipitate was filtered off, washed, and the filtrate and washings made up to 500 c. c. Two 25 c. c. portions of this were oxidized according to the Kjeldahl method, which gave the nitrogen of the protein-free extract (b).

Each of two 200 c. c. portions of this solution received enough sulphuric acid to make a 5 per cent sulphuric acid solution and boiled under a reflux condenser in a glycerine bath for two hours. On cooling, the hydrolysates were nearly neutralized with sodium hydroxide solution, made alkaline with magnesium oxide previously reduced to cream, and distilled in 1,000 c. c. Kjeldahl flasks, the distillate being received in an Erlenmeyer flask containing N/10 H_2SO_4 . The titration gave the ammoniacal nitrogen corresponding to the nitrogen of acid amides present in the extracts (c).

The residues which remained in the 1-liter Kjeldahl flasks were thoroughly extracted with boiling hot ammonia-free water, the extracts being filtered and washed with hot water. The black substance which, together with the magnesium oxide, remained on the filter was quantitatively transferred to a 500 c. c. Kjeldahl flask and oxidized according to Kjeldahl's method. The nitrogen thus obtained represented the humin nitrogen (d).

All of the filtrates and washings from the black substance were together concentrated on the water bath and made up to 100 c. c. In two 10 c. c. portions of this the total nitrogen was estimated according to Kjeldahl's method, and in another two 10 c. c. portions the amino nitrogen was determined according to the method of Van Slyke (22, 23, 24, 25), which yielded the nitrogen of amino acids present in the extracts (e).

To 50 c. c. of the remaining solution concentrated hydrochloric acid was added to a concentration of 20 per cent and boiled in a glycerine bath under reflux for 12 hours. The hydrolysate was next evaporated on the water bath to dryness in order to expel the hydrochloric acid, taken up with hot water and made up to 50 c. c., of which two portions of 10 c. c. each were oxidized according to the Kjeldahl method; in two other 10 c. c. portions the amino nitrogen was estimated according to Van Slyke's method. Subtracting from the result thus secured the amino nitrogen present in the solution prior to the hydrolysis was obtained the nitrogen of polypeptides present in the extracts (f).

The residual water-soluble nitrogen, which is made up of nitrogenous compounds other than those estimated above, constitutes the difference between the protein-free water-soluble nitrogen as obtained in (b) and the sum of the nitrogen secured as acid-amide nitrogen in (c), humin nitrogen in (d), amino nitrogen in (e), and peptide nitrogen in (f). The results obtained by the above methods are summarized in Table V.

TABLE V.—*Partition of the water-soluble nitrogen in the various seedlings*

[Results expressed in percentage of the water-soluble nitrogen of the seedlings]

No.	Conditions of germination				Acid-amide nitrogen	Humin nitrogen	Amino nitrogen	Peptide nitrogen	Residual nitrogen
	Duration	Substratum	Temperature	Treatment					
	<i>Days</i>		°		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	2	Paper.....	30	None.....	11.96	19.49	19.26	34.78	-----
2	2	do.....	30	do.....	10.91	19.53	22.39	33.35	-----
		Average.....			11.44	19.51	20.83	34.06	14.16
3	4	Paper.....	30	None.....	16.21	12.27	27.43	28.75	-----
4	4	do.....	30	do.....	14.91	-----	28.20	24.79	-----
		Average.....			15.56	12.27	27.82	26.77	17.58
5	8	Paper.....	30	None.....	18.90	10.52	-----	-----	-----
6	8	do.....	30	do.....	18.49	-----	-----	-----	-----
		Average.....			18.70	10.52	-----	-----	-----
7	4	Paper.....	25	Uspulun.....	13.90	8.57	28.06	34.52	-----
8	4	do.....	25	do.....	14.32	-----	28.51	-----	-----
		Average.....			14.11	8.57	28.29	34.52	14.51
9	8	Paper.....	25	Uspulun.....	18.32	6.54	28.89	29.52	-----
10	8	do.....	25	do.....	17.83	6.35	30.21	25.52	-----
		Average.....			18.08	6.45	29.55	27.52	18.40
11	11	Paper.....	25	Uspulun.....	17.87	9.22	31.70	25.98	-----
12	11	do.....	25	do.....	17.70	8.35	30.24	26.35	-----
		Average.....			17.79	8.78	30.97	26.17	16.29
13	15	Paper.....	25	Uspulun.....	16.71	7.06	31.86	20.98	-----
14	15	do.....	25	do.....	18.45	7.33	31.18	19.39	-----
		Average.....			17.58	7.20	31.52	20.19	23.50
15	-----	Ungerminated seed.....	-----	-----	11.47	26.81	9.53	35.03	-----
16	-----	do.....	-----	-----	11.78	16.58	9.51	-----	-----
		Average.....			11.63	21.70	9.52	35.03	22.12

[Results expressed in percentage of the total nitrogen of the seedlings]

17	2	Paper.....	30	None.....	2.98	4.86	4.80	8.67	-----
18	2	do.....	30	do.....	2.72	4.87	5.58	8.31	-----
		Average.....			2.85	4.87	5.19	8.49	3.53
19	4	Paper.....	30	None.....	6.42	4.86	10.87	11.39	-----
20	4	do.....	30	do.....	5.91	-----	11.17	9.82	-----
		Average.....			6.17	4.86	11.02	10.61	6.97
21	8	Paper.....	30	None.....	8.55	4.76	-----	-----	-----
22	8	do.....	30	do.....	8.36	-----	-----	-----	-----
		Average.....			8.46	4.76	-----	-----	-----
23	4	Paper.....	25	Uspulun.....	5.39	3.32	10.87	13.38	-----
24	4	do.....	25	do.....	5.55	-----	11.05	-----	-----
		Average.....			5.47	3.32	10.96	13.38	5.62
25	8	Paper.....	25	Uspulun.....	8.61	3.08	13.59	13.88	-----
26	8	do.....	25	do.....	8.38	2.99	14.21	12.00	-----
		Average.....			8.50	3.04	13.90	12.94	8.65

TABLE V.—*Partition of the water-soluble nitrogen in the various seedlings—Con.*

[Results expressed in percentage of the total nitrogen of the seedlings]

No.	Conditions of germination				Acid-amide nitrogen	Humin nitrogen	Amino nitrogen	Peptide nitrogen	Residual nitrogen
	Duration	Substratum	Temperature	Treatment					
	Days		°		Per cent	Per cent	Per cent	Per cent	Per cent
27	11	Paper.....	25	Uspulun..	7.67	3.96	13.61	11.15	-----
28	11	do.....	25	do.....	7.60	3.58	12.98	11.31	-----
		Average.....			7.64	3.77	13.30	11.23	6.99
29	15	Paper.....	25	Uspulun..	7.38	3.12	14.06	9.26	-----
30	15	do.....	25	do.....	8.14	3.24	13.76	8.56	-----
		Average.....			7.76	3.18	13.91	8.91	10.37
31	-----	Ungerminated seed.....			.49	1.16	.41	1.51	-----
32	-----	do.....			.51	.71	.41		-----
		Average.....			.50	.94	.41	1.51	.95

[Results expressed in percentage of the oven-dried seedlings]

33	2	Paper.....	30	None.....	0.051	0.084	0.083	0.150	-----
34	2	do.....	30	do.....	.047	.084	.096	.143	-----
		Average.....			.049	.084	.089	.147	0.061
35	4	Paper.....	30	None.....	.117	.088	.198	.207	-----
36	4	do.....	30	do.....	.107		.203	.179	-----
		Average.....			.112	.088	.201	.193	.127
37	8	Paper.....	30	None.....	.180	.100			-----
38	8	do.....	30	do.....	.176				-----
		Average.....			.178	.100			-----
39	4	Paper.....	25	Uspulun..	.096	.059	.194	.238	-----
40	4	do.....	25	do.....	.099		.197		-----
		Average.....			.098	.059	.196	.238	.100
41	8	Paper.....	25	Uspulun..	.158	.056	.249	.254	-----
42	8	do.....	25	do.....	.153	.055	.260	.220	-----
		Average.....			.156	.056	.255	.237	.158
43	11	Paper.....	25	Uspulun..	.147	.076	.260	.213	-----
44	11	do.....	25	do.....	.145	.068	.248	.216	-----
		Average.....			.146	.072	.254	.215	.134
45	15	Paper.....	25	Uspulun..	.137	.058	.261	.172	-----
46	15	do.....	25	do.....	.151	.060	.256	.159	-----
		Average.....			.144	.059	.259	.166	.193
47	-----	Ungerminated seed.....			.009	.021	.008	.028	-----
48	-----	do.....			.009	.013	.008		-----
		Average.....			.009	.017	.008	.028	.018

DISCUSSION AND INTERPRETATION OF RESULTS

When Table V is examined certain regularities stand out quite clearly. Thus, the acid-amide nitrogen rises fairly rapidly from 0.50 per cent in the ungerminated seed (Nos. 31 and 32) to 2.85, 6.17, and 8.46 per cent (Nos. 17 to 22), respectively, after 2, 4, and 8 days of germination, calculated on the basis of the total nitrogen. This is also true of the uspulun-treated seed, which after 4 days of

germination showed 5.47 per cent (Nos. 23 and 24), and after 8 days it rose to 8.50 per cent (Nos. 25 and 26). In the period from the eighth to the fifteenth day the acid-amide nitrogen remained almost constant (showing a slight diminution). The same regularities hold good when the latter is calculated to the water-soluble nitrogen or to the oven-dried seedlings.

The reverse, however, holds good for the humin nitrogen. Thus, while the ungerminated seed (Nos. 15 and 16) showed 21.70 per cent, it gradually decreased to 19.51, 12.27, and 10.52 per cent (Nos. 1 to 6) after 2, 4, and 8 days, respectively. The same is true of the uspulun-treated seed, which after 4 days of germination had 8.57 per cent (No. 7), but after 8 days had but 6.45 per cent (Nos. 9 and 10), calculated on the water-soluble nitrogen. In the period from the eighth to the fifteenth day the humin nitrogen showed a slight increase.

With regard to the amino nitrogen, it will be seen that from 9.52 per cent in the ungerminated seed (Nos. 15 and 16) it rose rather rapidly to 20.83 and 27.82 per cent after 2 and 4 days, respectively. Equally, the uspulun-treated seed showed an increase to 28.29, 29.55, 30.97, and 31.52 per cent after 4, 8, 11, and 15 days (Nos. 7 to 14), respectively, calculated on the basis of the water-soluble nitrogen. About the same relationship holds good when the amino nitrogen is calculated on either the total nitrogen or the oven-dried seedlings.

The figures for the peptide nitrogen show a reverse relationship. Thus, while the ungerminated seed (Nos. 15 and 16) had 35.03 per cent, it diminished to 34.06 and 26.77 per cent after 2 and 4 days, respectively, and in the case of the uspulun-treated seed it changed to 34.52, 27.52, 26.17, and 20.19 after 4, 8, 11, and 15 days, respectively, when calculated to the water-soluble nitrogen. The fact that the amino nitrogen rose at the same time that the peptide nitrogen diminished seems to indicate that the former increased at the expense of the latter.

The residual nitrogen, representing the difference between the total soluble nitrogen and the sum of the various nitrogenous compounds estimated, is rather fluctuating, as would be expected.

In Nos. 33 to 48 the results expressed in percentage of the oven-dried seedlings were given for the sake of completeness. These have not been recalculated to the original oven-dried seed.

The results in Table V are based upon the aqueous extracts (of the seedlings) which were successively treated with acetic and tannic acids. Although this treatment removes the proteins, it may not remove proteoses and peptones quantitatively (1, p. 609). If such be the case, the increase in amino nitrogen, on hydrolysis, as reported previously, could be due not only to the presence of polypeptides but also to that of proteoses and peptones. Hence, it seemed necessary, quantitatively, to remove any proteoses and peptones present prior to the hydrolysis. This was accomplished with the aid of phosphotungstic acid by the following method: On distilling off the ammonia, corresponding to the nitrogen of acid amides, the filtrate and washings from the magnesium oxide residue were concentrated on the water bath and made up to 100 c. c. On cooling, the solution was treated with 5 gms. of sulphuric acid and

30 c. c. of a solution containing 20 gms. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. After 24 hours, the precipitate was filtered off and washed with 200 c. c. of a solution containing 5 gms. of sulphuric acid and 2.5 gms. of phosphotungstic acid per 100 c. c., the washing being accomplished by rinsing the precipitate from filter into a beaker and returning to the filter three times. The washed precipitate was then oxidized according to the Kjeldahl method, yielding the nitrogen of any proteoses and peptones present, as well as of diamino acids.

The filtrate from the phosphotungstic acid precipitate was treated with calcium hydroxide to slight acidity, then with barium hydroxide to distinct alkalinity, saturated with carbon dioxide, heated, filtered on Büchner, and thoroughly washed with boiling hot ammonia-free water. The residual cake was extracted at least once more with hot water. The filtrates and washings from sulphate, phosphotungstate, and carbonate of calcium and barium were ordinarily saturated once more with carbon dioxide, and when clear concentrated in a vacuum, filtered, and made up to 100 c. c., of which 50 c. c. were used directly for the estimation of the amino nitrogen according to Van Slyke's method, and in the other 50 c. c. the amino nitrogen was estimated, on hydrolysis, as outlined above. By this method in which any proteoses and peptones present are removed, quantitatively, it was found that, on the average, after two days the seedling extract contained 22.08 per cent of amino nitrogen and 33.83 per cent of peptide nitrogen; after four days it contained 23.08 per cent of amino nitrogen and 30.60 per cent of peptide nitrogen, calculated on the basis of the water-soluble nitrogen. These figures reasonably approximate those obtained by the tannic-acid method alone, as a glance at columns 8 and 9 of Table V shows.

PROTEOLYTIC ENZYMES

The appearance of polypeptides, amino acids, and acid amides—well known disintegration products of proteins—in the aqueous extracts of the corn seedlings is to be attributed to the action of proteolytic enzymes. In view of the doubt expressed by some writers (2) as to whether these enzymes appear for the first time during the process of germination or are preexistent in the resting seed, it should be emphasized that the proteases are unquestionably preexistent in the ungerminated corn seed, since the latter was shown by the writer (11) to contain polypeptides and amino acids. This is further confirmed by the observation that the resting seed respire like the growing plant, though in a very much lesser degree (4), and it is for this reason that the decrease in weight shown by cereal grains during their storage is to be ascribed not only to loss of water but also to loss of carbon dioxide. The acid reaction which has been shown in this paper gradually to increase as germination of the corn seed progresses is a powerful aid to the proteases in their degradation of the proteins, since the action of proteolytic enzymes is known to be very much more intensive in an acid medium than in one that is neutral or alkaline (26, p. 223).

SUMMARY

By the process of germination, in the absence of light, the proteins present in the corn seed are rapidly undergoing disintegration whereby, within 8 days, up to 48 per cent of them are converted into water-soluble, diffusible nitrogen compounds.

The disintegration takes place through the activity of proteolytic enzymes which are to be considered as preexistent in the resting corn seed, since the latter was shown by the writer to contain polypeptides and amino acids, degradation products of protein.

From the beginning of corn germination up to the eighth day there is a steady increase in acid-amide nitrogen and a decrease in humin nitrogen. This is interpreted to mean that acid amides increase at the expense of certain amino acids which are known to contribute to the formation of humin nitrogen, such as tryptophane and tyrosine (5).

During the first eight days of corn germination there is also a steady rise in amino nitrogen and a diminution in peptide nitrogen. This is taken to mean that amino acids increase at the expense of polypeptides which, along with proteoses, are among the first degradation products of proteins.

The nitrogen distribution of the aqueous extracts of the etiolated corn seedlings, calculated to the water-soluble nitrogen, is as follows.

After 2 days: 11.44 per cent of amide nitrogen, 19.51 per cent of humin nitrogen, 20.83 per cent of amino nitrogen, and 34.06 per cent of peptide nitrogen. After 4 days: 15.56 per cent of amide nitrogen, 12.27 per cent of humin nitrogen, 27.82 per cent of amino nitrogen, and 26.77 per cent of peptide nitrogen. After 8 days (in uspulun-treated seedlings): 18.08 per cent of amide nitrogen, 6.45 per cent of humin nitrogen, 29.55 percent of amino nitrogen, and 27.52 per cent of peptide nitrogen.

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MINERAL COMPOSITION OF SUNFLOWERS GROWN FOR SILAGE¹

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INTRODUCTION

Sunflowers already have gained an important place among silage crops in the Pacific Northwest, chiefly because of their resistance to drought and frost and the high tonnage of forage produced per acre. Many high yields have been reported. In seasons of abundant rainfall 20 tons or more per acre have been obtained, while in seasons of deficient moisture approximately 10 tons are obtained. The fact that sunflowers usually result in high yields of forage per acre has given rise to the question by many agriculturists as to the effect of sunflowers on the following crop. Repeated observations have indicated that crops following sunflowers have been markedly decreased. To answer the common query of the farmers, "Do sunflowers deplete the soil nutrients?" a study was undertaken by the Idaho Agricultural Experiment Station to determine the amount of plant foods removed from the soil when sunflowers were grown under different systems of plantings. The Maryland station² has recently reported that sunflowers "draw about as heavily as other crops upon the nitrogen of the soil, less heavily than grain upon the phosphorus, and more heavily than other crops upon the potash. Owing to its heavy growth it exhausts unduly the plant food of the soil, and should therefore be grown in rotation with other crops."

Samples of sunflowers grown at the Idaho station in 1920 under different systems of plantings, and harvested at different stages of maturity, have been analyzed for their food constituents and the results reported in a previous publication.³ Quantities of each sample remained, and these offered an excellent opportunity to study the mineral content of sunflowers when harvested at different stages in their growth and under different systems of planting. For the complete plan of the experiment the reader is referred to the former publication.⁴

The sunflowers were grown on Palouse silt loam soil, which has the following general composition, as determined by Peterson:⁵

TABLE I.—Composition of Palouse silt loam soil^a

	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	Na ₂ O	K ₂ O	MAO	CO ₂	P ₂ O ₅	SO ₃	N	Or- ganic CO ₂
Surface soil----	67.66	14.90	4.44	2.89	1.31	2.72	1.96	0.0	0.04	0.160	Trace.	0.16	5.80
Subsoil-----	68.10	14.95	4.48	2.57	1.60	2.76	2.05	0.0	.03	.154	Trace.	.10	3.45

^a Figures indicate percentages.

¹ Received for publication Feb. 5, 1925; issued December 1925. Published by the permission of the director as Paper No. 35 of the Idaho Agricultural Experiment Station, 1925.

² ALLEN, E. W., BEAL, W. H., and FLINT, E. R. WORK AND EXPENDITURES OF THE AGRICULTURAL EXPERIMENT STATIONS, 1922, p. 28, 1924. (U. S. Dept. Agr., Office Exp. Sta.).

³ NEIDIG, R. E., and SNYDER, R. S. SUNFLOWER INVESTIGATIONS. Jour. Agr. Research 24:769-780. 1923.

⁴ NEIDIG, R. E., and SNYDER, R. S. Op. cit.

⁵ PETERSON, P. P. SOILS OF LATAH COUNTY, IDAHO. Idaho Agr. Exp. Sta. Bul. 107, 21 p., illus. 1918.

METHODS

The sunflowers were planted in rows 42 inches apart, the systems of planting differing in the distances apart (8 inches and 24 inches) in the row, and the number of plants (1, 2, 3, and 4) in the hill. The plants were collected and analyzed at five stages of growth.

STAGES SELECTED

The stages of maturity selected were as follows: First, when the bud was appearing on the top of the plant; second, when the first flower was about 3 inches in diameter but no seed had developed; third, just before the seeds of the first flower were in the dough stage; fourth, when the seeds of the first flower were well in the dough stage and the rays were just beginning to fall; fifth, when the seeds of the first flower were quite hard and its rays had fallen.

METHOD OF SAMPLING

Ten representative plants were collected, weighed, and measured, and then composited after cutting in a small silage cutter and mixing thoroughly. After complete desiccation, 250 grams were weighed out and carefully ashed. To prevent volatilization of the chlorides during ashing, the usual precautions were taken, that of leaching the residue after charring, then evaporating the leachings to a small volume, and incorporating this with the residue after burning the residue thoroughly in an electric muffle.

This residue is called the crude ash. Pure ash was obtained by dissolving the crude ash with hot dilute hydrochloric acid and filtering. The residue was again treated in the same manner and the filtrates combined and made up to a definite volume. Analyses of the pure ash were made in accordance with the methods outlined by the Association of Official Agricultural Chemists.⁶ In addition, total sulphur was determined on the finely ground unburned anhydrous material using the Parr Bomb method in contrast to the inorganic sulphur in the pure ash which was determined by the usual A. O. A. C. methods.

RESULTS

Table II contains data on the size and weight of stalks, the yield per acre, and the percentage of crude and pure ash for all the systems of plantings.

Table III contains data showing the mineral content of the ash of sunflowers expressed as percentage of the pure ash and also as percentage of anhydrous material for all systems of plantings and for all stages harvested.

⁶ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C. 1920.

TABLE II.—*Sunflower data*

No.	Stage	Num- ber in hill	Dis- tance apart in rows	Num- ber of stalks taken	Height	Average weight one stalk	Mois- ture	Yield per acre	Anhy- drous yield per acre	Crude ash	In- soluble residue	Pure ash
			<i>Inches</i>		<i>Ft. in.</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Tons</i>	<i>Tons</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
11005----	1	1	8	10	6 6	1.38	81.40			15.96	1.55	14.41
	2	1	8	10	6 1	1.53	83.44			14.12	1.86	12.25
	3	1	8	10	6 1	1.89	82.65			15.76	2.18	13.58
	4	1	8	10	6 1	2.05	81.40			14.00	1.56	12.44
	5	1	8	10	6 0	1.95	77.40	10.40	2.35	14.12	1.45	12.67
11008----	1	1	24	10	6 0	2.40	82.40			17.88	2.47	15.41
	2	1	24	10	6 0	2.80	81.93			17.31	3.05	14.25
	3	1	24	10	6 4	3.53	82.20			14.28	1.93	12.35
	4	1	24	10	6 6	4.34	82.53			13.28	1.61	11.67
	5	1	24	10	6 0	4.86	79.40	7.20	1.48	12.76	1.38	11.38
11010----	1	1	42	5	5 10	5.22	84.14			19.00	2.42	16.57
	2	1	42	5	5 5	5.16	85.53			16.48	1.91	14.57
	3	1	42	5	5 11	6.12	83.57			14.56	2.14	12.42
	4	1	42	5	5 11	8.02	83.80			13.52	1.70	11.82
	5	1	42	5	5 6	8.20	79.40	9.70	2.00	12.00	1.97	10.03
11011----	1	2	42	10	5 11	2.53	83.40			17.60	2.89	14.71
	2	2	42	10	6 2	2.73	83.67			15.76	2.30	13.46
	3	2	42	10	6 2	3.41	82.23			13.36	1.78	11.58
	4	2	42	10	5 10	4.35	82.07			13.36	1.77	11.59
	5	2	42	10	6 0	5.04	79.20	10.30	2.14	10.76	1.20	9.55
11002----	1	3	42	10	6 4	1.93	82.90			17.84	2.04	15.80
	2	3	42	10	6 2	2.05	81.87			14.32	1.58	12.73
	3	3	42	10	6 5	2.85	82.43			15.64	2.01	13.62
	4	3	42	10	6 4	4.06	83.00			14.67	1.70	12.97
	5	3	42	10	6 0	4.38	78.14	10.00	2.19	12.40	1.27	11.13
11013----	1	4	42	10	6 0	2.10	84.54			11.96	1.72	10.23
	2	4	42	10	6 1	2.02	82.40			14.56	1.94	12.61
	3	4	42	10	6 7	2.71	84.25			15.32	1.88	13.44
	4	4	42	10	5 10	2.73	79.70			13.36	1.49	11.86
	5	4	42	10	6 3	2.70	78.60	9.20	1.97	11.92	1.29	10.63

Table IV contains the data showing the amount of plant food in pounds removed per acre when sunflowers were cut for silage. The results are given for only the fifth stage, and are representative of the amount of plant food removed per acre by sunflowers when harvested for silage under field conditions. The results are stated as pounds of elements removed per acre by the different systems of planting, basing the calculations on the actual yields obtained. From the table it is seen that the yields are quite similar in all the systems of plantings, except Plot No. 11008, where the plants were spaced 24 inches apart in the row. This yield is low and should be attributed to differences in soil and moisture conditions rather than the spacing.

TABLE III.—Ash of Sunflower plants

No.	Stage	Number in hill	Distance apart in rows	Mineral content expressed in per cent of pure ash							Mineral content expressed in per cent of anhydrous material							
				Fe ₂ O ₃ and Al ₂ O ₃	Mn ₂ O ₄	CaO	P ₂ O ₅	K ₂ O	MgO	Inorganic sulphur	P ₂ O ₅	Fe ₂ O ₃ and Al ₂ O ₃	Mn ₂ O ₄	CaO	K ₂ O	MgO	Inorganic sulphur	Total sulphur
11005-----	1	1	Ins.	0.944	0.750	14.29	4.021	40.21	2.220	0.534	0.580	0.136	0.108	2.060	5.800	0.320	0.077	0.294
	2	1	8	1.200	1.436	16.36	4.279	38.61	4.556	.699	.524	.147	.176	2.006	4.731	.558	.086	.280
	3	1	8	1.546	.942	18.67	3.800	36.20	4.562	.696	.516	.210	.128	2.536	4.918	.620	.094	.270
	4	1	8	1.076	.981	16.30	3.879	33.36	4.483	.531	.495	.134	.122	2.028	4.154	.558	.066	.204
	5	1	8	1.065	1.121	15.90	3.621	27.48	5.160	.507	.459	.135	.142	2.016	3.480	.654	.064	.278
11008-----	1	1	24	1.220	.882	16.18	3.942	34.82	5.010	.582	.608	.188	.136	2.492	5.370	.772	.090	.408
	2	1	24	1.369	.828	19.13	5.056	33.91	5.040	.860	.720	.195	.118	2.724	4.835	.718	.122	.324
	3	1	24	.915	1.409	19.82	4.592	36.29	5.130	.582	.567	.113	.174	2.448	4.480	.572	.072	.306
	4	1	24	.848	1.457	19.15	4.816	34.50	3.990	.631	.562	.099	.170	2.234	4.025	.466	.074	.280
	5	1	24	.852	1.387	19.66	5.760	33.11	4.834	.862	.656	.097	.158	2.238	3.770	.550	.098	.326
11010-----	1	1	42	1.026	.482	26.83	4.228	35.55	4.740	.685	.701	.170	.080	4.448	5.896	.786	.113	.250
	2	1	42	.934	.700	26.20	4.680	38.58	3.308	.906	.682	.136	.102	3.818	5.620	.482	.132	.266
	3	1	42	1.192	.966	24.90	5.475	36.01	3.333	.862	.680	.148	.120	3.092	4.476	.414	.107	.218
	4	1	42	1.422	.677	22.50	5.418	40.75	2.708	1.002	.640	.168	.080	2.660	4.818	.320	.118	.234
	5	1	42	1.625	.877	26.83	6.327	34.60	8.510	.804	.635	.163	.088	2.692	3.472	.854	.081	.182
11011-----	1	2	42	1.516	1.006	22.46	4.610	38.30	5.140	.823	.678	.223	.148	3.304	5.636	.756	.121	.220
	2	2	42	1.284	.549	19.20	4.960	39.70	4.040	.921	.668	.173	.074	2.586	5.348	.544	.124	.456
	3	2	42	1.235	1.364	18.28	5.008	38.80	5.940	.870	.580	.143	.158	2.118	4.496	.688	.101	.260
	4	2	42	1.036	.811	20.33	5.108	38.80	4.622	.835	.592	.120	.094	2.358	4.498	.536	.097	.224
	5	2	42	1.256	1.006	20.46	5.658	39.11	7.250	.796	.540	.120	.096	1.954	3.736	.692	.076	.218
11002-----	1	3	42	1.158	.722	25.48	4.140	34.94	5.152	.919	.654	.183	.114	4.022	5.520	.814	.145	.330
	2	3	42	.935	.833	20.58	4.467	38.80	4.505	.845	.569	.119	.106	2.620	4.940	.574	.107	.332
	3	3	42	1.079	.983	18.10	4.850	41.28	4.840	.723	.661	.147	.134	2.466	5.622	.660	.098	.306
	4	3	42	1.041	.740	15.81	5.260	34.48	4.500	.740	.682	.135	.096	2.050	4.472	.584	.096	.280
	5	3	42	1.005	.828	19.16	5.480	33.35	4.165	.741	.610	.112	.092	2.134	3.715	.464	.082	.306
11013-----	1	4	42	1.515	1.212	20.79	6.150	48.05	7.175	1.171	.629	.155	.124	2.126	4.918	.734	.120	.174
	2	4	42	1.332	.539	16.75	5.900	38.65	6.120	.996	.744	.168	.068	2.112	4.876	.772	.126	.242
	3	4	42	1.272	.982	18.40	5.120	40.65	5.520	.926	.688	.171	.132	2.472	5.464	.742	.124	.228
	4	4	42	1.483	1.011	18.46	6.060	39.38	2.629	.978	.719	.176	.120	2.190	4.674	.312	.116	.220
	5	4	42	1.138	.903	20.00	5.905	40.61	3.970	.730	.628	.121	.096	2.128	4.320	.422	.078	.170

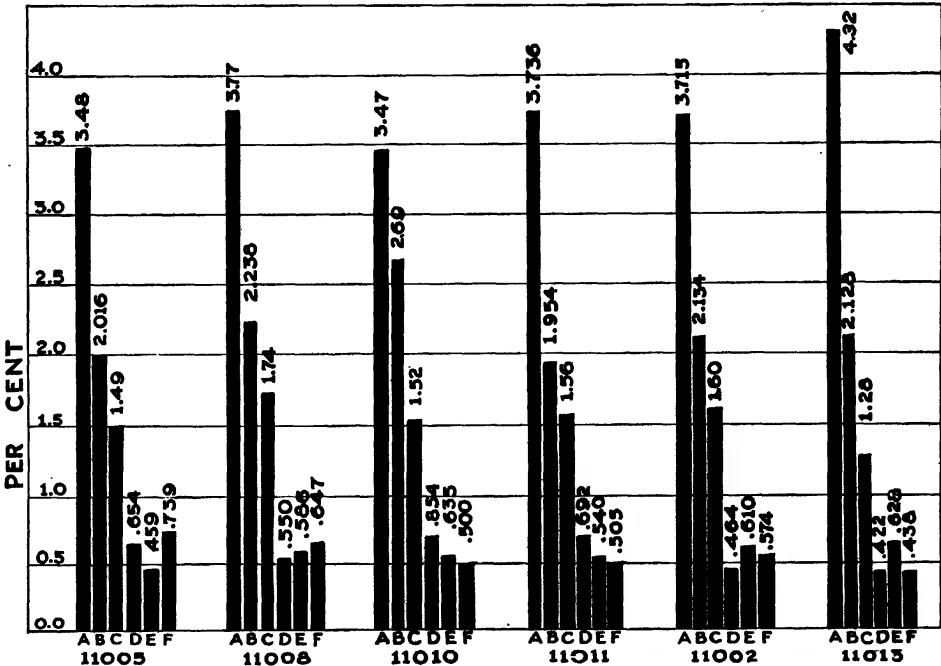


FIG. 1.—Percentage composition of sunflowers, expressed on the anhydrous basis for the different systems of planting. (Fifth stage only.) A=K₂O; B=CaO; C=N; D=MgO; E=P₂O₅; F=other elements

The variations observed in yields are due for the most part to the uneven moisture and fertility conditions, because of the uneven contour of the land. Figure 1 represents graphically the percentage of mineral elements in the ash of sunflowers from all systems of planting, based on the anhydrous material. Since the majority of the systems of plantings yielded sunflowers to the amount of approximately 10 tons, a graphic representation of the pounds of mineral elements removed from 1 acre by sunflowers when cut for silage is given in Figure 2.

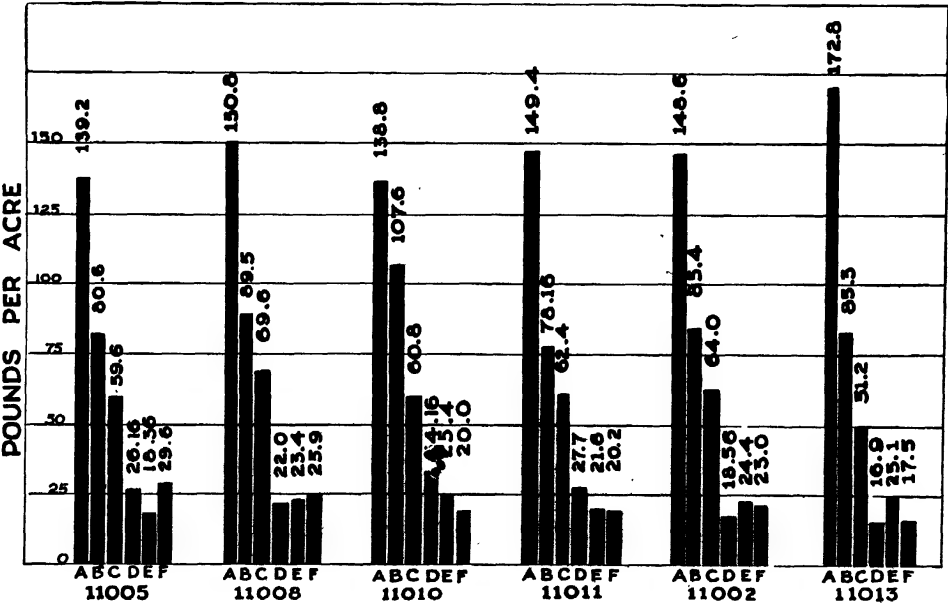


FIG. 2.—Pounds of elements removed per acre by a 10-ton per acre crop of sunflowers. A=K₂O; B=CaO; C=N; D=MgO; E=P₂O₅; F=other elements

In calculating the pounds removed per acre, the moisture percentage taken for all stages was arbitrarily placed at 80 per cent, which is relatively near the average moisture for sunflowers when cut at this stage for silage.

TABLE IV.—Analysis of ash of sunflowers—actual pounds of elements removed per acre

[Fifth stage only, i. e., when seeds of first flower were quite hard and its rays had fallen]

No.	Number in hill	Distance apart in rows	Crude ash	In-soluble residue	Pure ash	P ₂ O ₅	Fe ₂ O ₃ and Al ₂ O ₃	Mn ₃ O ₄	CaO	K ₂ O	MgO	Inorganic sulphur	Total sulphur	Weight anhydrous sunflowers per acre
		Inches												Pounds
11005.....	1	8	663.8	68.3	595.5	21.58	6.35	6.68	94.77	163.6	30.74	3.02	13.07	4,701
11008.....	1	24	378.5	40.96	337.5	19.46	2.88	4.69	66.38	111.8	16.31	2.91	9.67	2,966
11010.....	1	42	479.5	78.76	400.8	25.37	6.51	3.52	107.57	138.7	34.13	3.22	7.27	3,996
11011.....	2	42	461.1	51.63	409.5	23.14	5.14	4.11	83.73	160.1	29.65	3.26	9.34	4,285
11002.....	3	42	542.1	55.70	486.4	26.67	4.90	4.02	93.30	162.4	20.29	3.61	13.38	4,372
11013.....	4	42	469.4	50.88	418.5	24.73	4.76	3.78	83.80	170.1	16.62	3.06	6.69	3,938
Average.....			499.1	57.71	441.4	23.50	5.09	4.47	88.28	151.1	24.62	3.18	9.90	4,043

Figure 3 contains a graphic comparison of the average analysis of 55 sunflower plants analyzed by the writers, and of 5 corn plants calculated from data published by Latshaw.⁷ In addition a comparison of the amount of elements removed from an acre of sunflowers and corn is made, assuming the yield of each crop to be 10 tons and the moisture percentage of sunflowers 80 per cent, while corn is calculated at 70 per cent. Latshaw's analyses are used for the basis of the corn calculations.

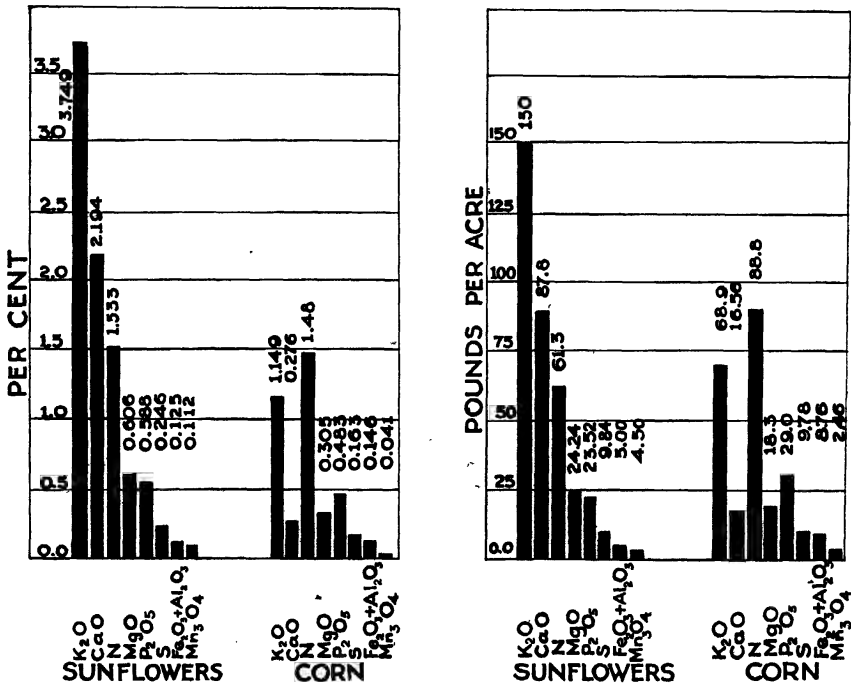


FIG. 3.—Comparison of the percentage composition and pounds of elements removed per acre by 10-ton per acre crops of sunflowers and corn

A comparison of the percentage composition of mineral elements in sunflowers when grown under the different systems in this investigation shows that regardless of the spacings or type of planting, the percentage composition is approximately the same. The greatest variation is found in plot 11013, which contained four plants per hill. These plants drew the heaviest upon the potash of the soil and gave the lowest percentage of nitrogen. An examination of this particular group of plants through the five stages of growth shows a limited growth in the later stages. This may be due to the inability of the roots to feed over as large an area in the hill system with four plants as when spaced at stated distances in the row.

COMPARISON OF ELEMENTS IN SUNFLOWERS AND CORN

Sunflowers draw very heavily on potassium and calcium and to some extent on magnesium. They draw much more heavily on these bases than does corn. As to the other elements, sunflowers and corn take about the same amounts out of the soil.

⁷ LATSHAW, W. L., and MILLER, E. C. ELEMENTAL COMPOSITION OF THE CORN PLANT. Jour. Agr. Research 27: 845-860, illus. 1924.

Figure 3 shows the difference in the mineral composition of sunflowers and corn when expressed as percentage of the dry material and when expressed as pounds removed per acre, assuming an arbitrary yield of 10 tons for each crop. The moisture content of sunflowers was placed at 80 per cent and corn at 70 per cent, which is near the average figure for each crop.

Comparing the percentage composition it is seen that sunflowers contain more than three times as much potassium oxide, eight times as much calcium oxide, and twice as much magnesium oxide, as does corn. The percentage composition in respect to phosphorus pentoxide, sulphur, and other elements is about the same for both crops, there being slightly greater percentages in sunflowers than in corn.

In spite of the fact that a 10-ton yield of sunflowers, as cut for silage, yields only 4,000 pounds of anhydrous material, while 10 tons of corn yields 6,000 pounds, it is found that the amount of bases removed by sunflowers is still much greater than is removed by corn as cut for silage. The two elements showing the greatest differences are potassium and calcium, 150 pounds of potassium oxide and 87.8 pounds of calcium oxide being removed by sunflowers as against 68.9 pounds of potassium oxide and 16.6 pounds calcium oxide by corn. Magnesium oxide is about $1\frac{1}{3}$ times greater, there being 24.24 pounds removed by the sunflowers and 18.3 pounds by the corn. Sulphur is removed in approximately the same amounts by both crops, sunflowers removing 9.84 pounds and corn 9.78 pounds per acre. More phosphorus pentoxide is removed by corn than by sunflowers, the amounts being 29 pounds and 23.5 pounds, respectively.

While the percentage of nitrogen is approximately the same in sunflowers and corn, the amount removed by the 10-ton crop of corn is greater than that removed by a corresponding crop of sunflowers, due to the greater amount of anhydrous material. A 10-ton yield of corn contains 88.8 pounds nitrogen as against 61.3 pounds for the similar yield of sunflowers.

The reason that low yields of crops sometimes follow sunflowers may be due to the heavy draft which a sunflower crop makes on the soluble minerals of the soil, a heavy crop of sunflowers requiring especially a large supply of potassium and calcium. It is readily seen, therefore, that sunflowers, when grown, should be included in a rotation.

SUMMARY

The composition of the ash of sunflowers has been determined for different systems of plantings and for different stages of growth.

A comparison of the minerals removed by 10-ton crops of sunflowers and corn is given.

Sunflowers draw more heavily than corn upon certain soil elements, especially potassium and calcium.

INFLUENCE OF FIELD-PEA RATIONS ON THE QUALITY OF PORK¹

By J. E. NORDBY, *Assistant Animal Husbandman, Department of Animal Husbandry*, and ROBT. S. SNYDER, *Associate Chemist, Department of Agricultural Chemistry, University of Idaho*

INTRODUCTION

More and more during recent years, field peas have been replacing summer fallow in Idaho. It seems quite probable that they will be grown to increasing extent as their value in rotation systems becomes more generally appreciated.

Two types of feeding field peas have come into general use among farmers in the State: First, "hogging-off"; and, second, feeding in the dry lot, especially cull peas that are not suitable for seed. In work done at the Idaho station² during the last eight years, an average of 406 pounds of pork have been produced per acre by the "hogging-off" method, while in the dry lot less than 400 pounds of peas have been required per 100 pounds of gain. In some cases unthreshed pea vines have been harvested and used for either wintering or finishing hogs.

As a result of their general use, many questions have come to this station relative to the value of peas with respect to their specific influence on the quality of pork produced when fed alone, or in combination with standard concentrates. A few complaints have been made that hogs finished on peas were discriminated against at market because "they lacked the firmness which characterizes hogs fed on corn or barley and that they killed out soft carcasses."

Reference is made to this problem by Shaw³ who concludes that "peas are superior to corn as a food for pigs at any time prior to the fattening season; hence they may be fed to swine more freely, but in no instance should they form the sole ration before the finishing period begins. 'During the fattening period they are unexcelled when fed as the sole grain food. They promote growth, while they fatten in excellent form, and they furnish a sweet, firm, and excellent quality of pork.' " Day⁴ refers to pea meal as "a valuable food," but says it "should never be fed alone. * * * Peas are noted for the excellent quality of bacon which they produce." Toole and Knox⁵ include peas as a desirable forage for developing bacon hogs, and Grisdale⁶ says "peas are 'undoubtedly of very high value as a feed for the production of good, firm bacon, and for young pigs and breeding stock of all classes at practically all times. They should,

¹ Received for publication Feb. 5, 1925; issued December, 1925. Published with approval of the director as paper No. 31 of the Idaho Agricultural Experiment Station. The writers are indebted to C. W. Hickman, head of the Department of Animal Husbandry, and Ray E. Neidig, head of the Department of Agricultural Chemistry, University of Idaho, for valuable suggestions in the planning and carrying out of the work.

² GONGWER, R. E. FIELD PEAS FOR PORK PRODUCTION. Idaho Agr. Exp. Sta. Bul. 125, 8 p., 1921. Unpublished data.

³ COBURN, F. D. SWINE IN AMERICA. p. 356. New York and London. 1909.

⁴ DAY, G. E. BACON PRODUCTION. Ontario Agr. Col. and Exp. Farm Bul. 129, 23 p., illus. 1903.

⁵ TOOLE W., and KNOX, R. G. BREEDING, GROWING AND FINISHING "THE BACON HOG." Ontario Agr. Col. and Exp. Farm Bul. 299, 10 p., illus. 1923.

⁶ COBURN, F. D. Op. cit. p. 357.

however, never be fed alone, and should always be ground. Pigs fed on pea meal alone do not thrive, do not get fat, and produce a very inferior quality of meat, dry and hard.' ⁷ Peas, largely in the form of meal, have a rather important part in hog feeding in Europe where bacon production is a specialty.

PURPOSE

The purpose of this investigation is to study the quality of pork produced from field peas exclusively and compare it with pork obtained from pea-barley, barley-tankage, and corn-tankage rations, from the standpoint of shrinkage in the curing processes, melting points, and iodine values.

PLAN

Two methods of feeding were adopted. In part 1 the hogs were confined to the dry lot. In part 2 a preliminary forage period preceded the dry-lot feeding.

PART I

Thirty-two thrifty, uniform, Duroc-Jersey shotes, averaging 120 pounds, were divided into 4 lots, and fed for 76 days (February 23 to May 9, 1923), on the following rations:

Lot I, cracked peas alone.

Lot II, cracked peas 3, barley 7.

Lot III, rolled barley 15, tankage 1.

Lot IV, cracked corn 9, tankage 1.

In addition to these rations, all lots received, with the evening feed, 2 pounds of the following mineral mixture: 100 pounds bone meal, 100 pounds charcoal, 50 pounds common salt, 50 pounds sulphur, 25 pounds copperas.

PART 2

Forty-six well-developed, uniform, Poland-China and Duroc-Jersey shotes, averaging 98.5 pounds, were divided into 4 lots. A forage period of 30 days was introduced prior to the dry-lot feeding, according to the following plan:

Lot I, hogging-off, 0.85 acre peas.

Lot II, hogging-off, 0.95 acre peas, and in addition a 1 per cent ration of rolled barley.⁷

Lot III, alfalfa forage and a full ration of rolled barley 15, tankage 1.

Lot IV, alfalfa forage, and a full ration of cracked corn 12, and tankage 1.

Feeding was then continued in the dry lot, as follows:

Lot I, cracked peas alone.

Lot II, cracked peas 7, rolled barley 2.

Lot III, rolled barley 14, tankage 1.

Lot IV, cracked corn 10, tankage 1.

⁷ Eleven hogs were used in lots I, III, and IV. Two additional hogs were used in lot II in order to hog off the peas at the same rate as in lot I.

RESULTS

All lots in both parts of this series were provided with comfortable accommodations. Weight records were made of each pig every 14 days. The initial and final weights of each test represent the average weight of three consecutive days, the second of which marks the beginning and close of the test.

The hogs were marketed at a packing plant in Moscow, Idaho. Careful records were kept on each lot as to dressing percentages, shrinkage in the cooler, and gains or losses in the curing and smoking processes. Physical observations were made as to the quality of pork.

For the determination of the melting points and iodine values, samples of fat were taken from the middle of the back and from the leaf after the carcasses were chilled. Each sample was rendered separately and poured into small glass bottles. All chemical analyses were made according to the Official Methods of the Association of Official Agricultural Chemists.⁸

The composition of all feeds used was as follows:

TABLE I.—*Composition of feeds*

Feed	Mois- ture	Ash	Crude protein	Crude fiber	Nitrogen- free extract	Fat
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Blue Prussian peas.....	9.87	4.17	22.33	4.31	57.38	1.94
White winter barley.....	9.65	2.72	12.26	6.10	67.31	1.96
Yellow Dent corn.....	10.97	1.55	10.07	2.16	73.17	2.08
60 per cent digester tankage.....	7.02	17.18	57.79	2.62	2.70	12.69

Tables II, III, and IV are introduced to show the average daily gain, the amount of feed per 100 pounds gain, and the dressing percentages of each lot under the two types of feeding.

An economy of gain is registered in the peas-following-forage lot in Table IV as compared to the pea lot in Table III. A forage period has the same effect in the case of all other lots.

The dressing percentages of the pea-fed lots were somewhat lower than those in the other lots. It appeared to be the tendency of the pea-fed hogs to grow rather than to fatten, which will account for the lower dressing percentages. It will be noted that the feed requirement for 100 pounds of gain is in favor of the pea-fed lots, and that an unusually low amount was required in Lot I, Table III. The pea-fed hogs did not display the vigorous appetite at all times that was evident in the lots receiving mixed rations. In all cases, the feed requirement per 100 pounds of gain was quite satisfactory.

⁸ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C. 1920.

TABLE II.—*Comparison of peas, barley, corn, and tankage in the following combinations for finishing hogs (part 1)*

Ration	Lot 1: Peas	Lot 2: Peas 3, barley 7	Lot 3: Tankage 1, barley 15	Lot 4: Tankage 1, corn 9
Average daily gain (pounds).....	1. 36	1. 41	1. 31	1. 41
Pounds of feed per 100 pounds gain.....	378. 40	410. 90	438. 34	409. 52
Dressing percentages.....	77. 03	80. 46	78. 57	81. 13

TABLE III.—*Hogging-off peas as the only ration: Hogging-off peas with 1 per cent barley, and alfalfa forage with full grain ration (part 2)*

Ration	Lot 1: 0.85 acre peas	Lot 2: 0.95 acre peas, barley 1 per cent ration	Lot 3: Alfalfa forage, barley 15, tank- age 1	Lot 4: Alfalfa forage, corn 12, tankage 1
Average daily gain (pounds).....	0. 96	<i>Per cent</i> 1. 07	1. 17	1. 30
Pounds of supplementary feed per 100 pounds gain.....	-----	101. 20	400. 30	360. 80

TABLE IV.—*Forage lots continued on the following rations (part 2)*

Ration	Peas	Peas 7, barley 2	Barley 14, tank- age 1	Corn 10, tankage 1
Average daily gain (pounds).....	1. 49	1. 64	2. 02	2. 01
Pounds of feed per 100 pounds gain.....	362. 20	393. 40	411. 30	414. 70
Dressing percentages.....	74. 00	74. 20	79. 10	78. 50

COOLING AND CURING RESULTS

The carcasses were cooled 48 hours in a direct-expansion type of cooler at a temperature of 35° F. It was deemed sufficient to keep records through the curing and smoking processes on the sides (bellies) only. The bellies in part 1 were placed in the sweet-pickle cure (salometer reading 60) for 33 days, then removed and drained for 10 hours before weighing into the smoke. After smoking 14 hours at a temperature of from 90° to 110° F. they were cooled for 8 hours and weighed.

In part 2, the bellies were cured by the dry-salt process for 25 days. After removing from the salt they were soaked 4 hours and allowed to drain 10 hours before weighing into the smoke. From this point the procedure was the same as in part 1.

A summary of results, as shown in Table V indicates very small difference between the various lots in either part 1 or part 2. It would seem rather difficult to point to any significant variation in these tables which might tend to indicate any appreciable difference in the lots involved.

TABLE V.—*Summary of results in the cooling, curing, and smoking processes*

Lot No.	Part 1.—Sweet-pickle cure					Part 2.—Dry-salt cure				
	Fresh chilled weight	Cooled smoked meat	Loss			Fresh chilled weight	Cooled smoked meat	Loss		
			Cooler	Through cure	Through smoke			Cooler	Through cure	Through smoke
	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	200	191.5	—2.68	7.50	—4.25	152	138	—2.65	—2.63	—9.21
2.....	195	187.0	—3.08	7.69	—4.10	171	155	—2.82	—2.34	—9.35
3.....	154	148.0	—2.83	7.79	—3.89	225	205	—3.22	—2.22	—8.88
4.....	215	203.0	—2.80	6.97	—5.58	297	270	—2.95	—2.35	—9.09

MELTING POINTS OF LEAF AND BACK FATS

In Table VI only the average melting points of the various lots are shown. The individual variance from the average, not only in the melting points, but in the iodine values also, was so small that it was not deemed worth while to give each result. In this work the temperature at which the fats became transparent was taken as the melting point.

TABLE VI.—*Melting points of back and leaf fats*

Lot No.	Part 1		Part 2	
	Back fat	Leaf fat	Back fat	Leaf fat
	° C.	° C.	° C.	° C.
1.....	40.90	48.16	41.2	47.5
2.....	41.97	48.50	42.4	48.4
3.....	42.63	48.63	44.6	50.8
4.....	41.06	48.05	44.0	49.4

Very little difference is to be noted in the melting points of the fats from the various lots.

In part 1 there is a variance of only 0.58° in the leaf fat and 1.73° in the back fat. In part 2 the difference is slightly greater, there being a variance of 3.3° in the leaf fat and 3.4° in the back fat. In both part 1 and part 2 the melting points in the barley and tankage fed lots were consistently higher than in the other lots. This difference, however, is not sufficient to be of any great significance.

IODINE VALUES

The iodine values were run according to the Hanus method. From these the percentage of olein was calculated according to the table given in Lewkowitsch.⁹

⁹ LEWKOWITSCH, J. CHEMICAL TECHNOLOGY AND ANALYSIS OF OILS, FATS, AND WAXES. Ed. 4, rewritten and enl., v. 3, p. 176 London. 1909.

TABLE VII.—Iodine values of leaf and back fats

Lot No.	Part 1				Part 2			
	Back fat		Leaf fat		Back fat		Leaf fat	
	Iodine value	Per cent olein	Iodine value	Per cent olein	Iodine value	Per cent olein	Iodine value	Per cent olein
1.....	66.08	73.36	55.3	61.39	65.1	72.27	57.5	63.83
2.....	61.5	68.24	55.68	61.79	63.7	70.72	54.6	60.57
3.....	65.9	73.15	55.76	61.90	62.4	69.27	52.3	58.06
4.....	64.6	71.71	56.84	63.09	62.0	68.83	53.4	59.28

Here, as in the determination of the melting points, very little difference can be noted between the various lots. In part 1 there is a variance of only 1.7 per cent olein in the leaf fat and 5.12 per cent in the back fat. In part 2 the widest variation was 5.77 per cent olein in the leaf fat and 3.44 per cent in the back fat.

DISCUSSION OF THE RESULTS

The individual variation in gains was greater in the pea-fed lots. Some of the hogs in the lots took to peas readily and did not tire of them, while others showed a tendency to go off feed frequently, influencing the uniformity of gains in the lots. Due to these variations, the pea-fed lots did not show as uniformly high a finish as did the other lots, and, in addition, tended to be paunchy. Both of these conditions somewhat lowered the dressing percentages. Barley, added to the pea ration, tended to overcome these conditions, the hogs having a better appetite, being less paunchy, and having a higher dressing percentage.

In the physical observation of carcasses in both part 1 and part 2 it was impossible to distinguish carcasses in one lot from those in another lot when of the same finish. The best carcasses in the pea-fed lots appeared in every respect equal to those in lots III and IV.

It would seem from these data and observations that one need not fear the production of soft pork when feeding peas, either alone or in combination. Commercial peas are ordinarily too high in price to be fed as the only grain ration. However, cull peas, which are quite comparable in feeding value to some of the standard feeds, are often available at reasonable figures.

Hogs fed on peas alone, either in the dry lot or on forage, tend to grow rather than fatten, and for this reason finish somewhat slower than when fed standard rations.

The feed requirement for 100 pounds of gain is in favor of the pea-fed lots, even though at times the pea-fed hogs did not have as vigorous an appetite as was evident in the other lots.

The quality of pork, when judged by physical observations of the carcasses, shrinkages in the curing process, melting points, and iodine values, tends to show that peas, when fed alone or with barley, compare favorably with such standard rations as barley and tankage, and corn and tankage.

GERMINATION OF FROZEN AND NONFROZEN WHEAT HARVESTED AT VARIOUS STAGES OF MATURITY¹

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INTRODUCTION

In the northern part of the United States, and especially in the Rocky Mountain region and in western Canada, where the growing season is relatively short, spring wheat is frequently subjected to temperatures below freezing at immature stages of growth. Kernels threshed from frosted wheat may be recognized by the blistered or wrinkled appearance of the outer layers of the bran and by their green pigmentation. Usually the more immature the wheat at the time of frost the greater the number of green kernels. The green kernels may not be blistered.

Lugger (10)² attempted to distinguish between frosted and frozen wheat. He states:

We see at once the important difference between frosted wheat and frozen wheat. In the former germination may take place in most cases; in the latter never, because the living substance of the seed, the protoplasm, is dead and can not be resurrected by any known means.

Harper (7) states more clearly the difference, saying:

Frozen wheat is badly shrunken, has lost the normal translucent amber color, is of an opaque bronzed appearance, and has had the composition of its chemical constituents changed as well as the internal structure of its cells destroyed.

Blistered (frosted) wheat retains the normal amber color, but has in many cases more gluten and protein and less starch than sound wheat, and is injured for milling on account of the bad condition of the hull. Only in extreme cases has there been any injury to the germ and its surrounding food, so that it is all right for seed, if well cleaned, except in some cases where frost has caused the injury because of the tardiness of the wheat to mature.

Green (5) tested the germination of frosted wheat in soil in the greenhouse. He found that the germination in the series of graded frosted wheats which he investigated ranged from 68 to 92 per cent. The lowest value found for 8 samples of wheat classed as chicken feed was 42 per cent. Some of these samples had been injured by both frost and rust.

Keffer (8) examined 13 samples of wheat. He determined the percentages of "plump," "slightly shriveled," and "much shriveled" kernels in each sample. Seven samples which contained 50 per cent or more of much shriveled kernels and averaged 72 per cent gave an average germination of 72 per cent in soil and 82 per cent between moist papers. Three samples with a content of much shriveled kernels ranging from 29 to 47 per cent gave an average germination of about 76 per cent in soil and 91 per cent between moist papers. The wheat which he reported as normal contained 5 per cent of much shriveled kernels and gave a germination of 82 per cent in soil and 99 per cent between moist papers.

¹ Received for publication May 2, 1925; issued December, 1925. Published with the approval of the director of the station.

² Reference is made by number (italic) to "Literature cited," p. 1188.

Atkinson, Whitlock, and Jahnke (1) investigated the seed value of frosted wheat. They separated into two parts each of 32 samples of wheat which contained a large percentage of frosted kernels, one containing only kernels showing frost injury and the other containing kernels showing no frost injury. The germination of these samples was tested in the field. The average germination of the frosted samples was 75 per cent and that of the nonfrosted 78 per cent. Their results show a greater number of heads per row and a higher yield from the frosted kernels. They state:

The difference is not great enough to be very significant, yet it shows that kernels showing frost injury are not necessarily worthless for seed. It should be understood that grain may be entirely destroyed by frost, so far as its seed value is concerned, but this test suggests that all grain need not be discarded for seed purposes because it shows frost injury. The only safe plan is to have it tested before deciding either to use or discard it.

Atkinson and Jahnke (2) carried out further investigations on the germination of frosted wheat along somewhat the same line. The report of their experiments indicates that the frosted wheat showed a less germination than the nonfrosted wheat separated from the same samples.

Miss Lute (11) states that frosted wheat from San Luis Valley, Colo., showed low germination, but gives no definite data on the subject.

The effect of freezing temperatures produced by artificial means on the germination of seeds has been studied by a number of investigators. Detmer (4) states that air-dry wheat kernels can be subjected to temperatures of -5° to -10° C. without injuring their germination, while if the turgid kernels are subjected to these temperatures the germination is injured. Thiselton-Dyer (13) found that air-dry wheat kernels could be subjected to the temperature of liquid hydrogen for 1 hour without injuring their germination. Becquerel (3) subjected air-dry wheat kernels to liquid air for 130 hours and found that germination was unimpaired after this treatment.

EXPERIMENTAL DATA

A part of a 1923 crop of Marquis wheat was purchased from a farmer living near the Montana Agricultural Experiment Station. This wheat was grown under a system of dry farming. Portions of the wheat were harvested at intervals of two to four days during the development of the kernel. In order to restrict the amount of material flowing into the kernel after harvest, only the heads were gathered. Enough heads were obtained at a time to fill six 24-pound flour sacks. Three of these sacks were placed in the hardening room of an ice-cream manufacturing plant for 48 hours, after which time they were spread on the floor of a large room to dry. The other three sacks picked at the same time were at once spread on the floor to dry. The temperature of the hardening room ranged between -20° and -28° C. After the heads became dry they were threshed.

The moisture content of the kernels was determined at the time of harvest. The values obtained are given in Table I. The weight per kernel was calculated from the weight of 1,000 kernels. Typical frozen and nonfrozen kernels at four stages of development were photographed (pl. 1). Estimating the age of the kernels from the

data given by Sharp (12) for the development of Marquis wheat obtained in a similar experiment the previous year, it is probable that the kernels in samples 131 and 132 (see Table I) are about 13 days old. In the previous year (Table I of the paper by Sharp) kernels 13 days old contained 70.7 per cent moisture and weighed 7.9 mgms., while sample 131 contained 69.4 per cent moisture at the time of harvest and the dry weight per kernel was 7.7 mgms. This estimated age of the kernel is probably correct to within one or two days. The kernels representative of sample 131, Plate 1, A, have the yellow color of mature wheat, the kernels from the sample 132, Plate 1, B, which was collected at the same stage of maturity but which was frozen before drying, are entirely green in color. Samples 137 C and 138 D in Plate 1 were collected 12 days later and therefore are approximately 25 days old. The frozen kernels, 138, still show some green color, and blistering of the outer bran layers is apparent. Samples 141, Plate 1, E, and 142, Plate 1, F, were approximately 29 days old; at this stage of development no green kernels were found in the frozen sample; the blistering of the outer layers of the bran was greatest during this period of development. Sample 149, Plate 1, G, and sample 150, Plate 1, H, were approximately 38 days old, and blistering of the frozen kernels was still marked. Samples 149 and 150 were collected five days after the time the farmer considered the best for cutting the main part of his field of wheat. Thus the farmer decided to cut his wheat when the kernels were approximately 33 days old. Sample 152 also showed marked blistering. It is thus apparent that blistering may be produced by severe freezing even when the moisture content of the wheat is 34 per cent or less. Possibly if the freezing temperature had been less severe blistering might not have occurred at such a low moisture content. Farmers frequently believe that their wheat is safe from frost damage as soon as it is in the shock. Whether or not wheat is safe from frost damage probably depends on the moisture content of the kernel and the freezing temperature and its duration. Additional data on this series of wheat are given by Sharp (12) and will not be repeated here.



Kernels of frozen and nonfrozen wheat harvested at various stages of maturity

- A.—Sample No. 131, nonfrozen, approximate age, 13 days
 B.—Sample No. 132, frozen, approximate age, 13 days
 C.—Sample No. 137, nonfrozen, approximate age, 25 days
 D.—Sample No. 138, frozen, approximate age, 25 days
 E.—Sample No. 141, nonfrozen, approximate age, 28 days
 F.—Sample No. 142, frozen, approximate age, 28 days
 G.—Sample No. 149, nonfrozen, approximate age, 38 days
 H.—Sample No. 150, frozen, approximate age, 38 days

Germination tests as described by Whitcomb (14) were made on November 24 and December 21, 1923, and November 22, 1924, by the alternating-temperature method. This test, as carried out, consisted in placing 100 kernels between pieces of moist blotting paper and keeping them for 18 hours at 20° C., and then raising the temperature to 30° C. for six hours, and then lowering it to 20° C. for 18 hours, etc. On December 21, 1923, the germination was determined also by the ice-box method. In this method 100 kernels were placed between moist blotting papers, and kept continuously in the ice box at 4° to 6° C. for five days, at the end of which time the wheat was treated by the alternating-temperature method as described above. The tests were all carried out in duplicate. The results are given in Table I.

TABLE I.—*Marquis wheat: Germination of frozen (temperature —20° to —28° C.) and nonfrozen wheat harvested at various stages of maturity*

[Harvest began Aug. 9, 1923, when the kernels were about 13 days old. The germination tests were all run on duplicates of 100 kernels. Where 0.5 per cent occurred in the average it was added as 1 per cent]

Laboratory No.	Approximate age of kernel	Moisture at time of harvest	Weight per kernel, moisture-free	Germination			
				Nov. 24, 1923, alternating temperature 20° to 30° C., 9 days	Dec. 21, 1923		Nov. 22, 1924, alternating temperature 20° to 30° C., 6 days
					Alternating temperature 20° to 30° C., 6 days	Ice box, 5 days, alternating temperature 3 days	
	Days	Per cent	Mgs.	Per cent	Per cent	Per cent	Per cent
131, nonfrozen.....	13	69.4	7.7	98	95	99	91
132, frozen.....	13	69.4	-----	1	2	0	0
133, nonfrozen.....	17	62.5	14.0	98	99	99	96
134, frozen.....	17	62.5	-----	3	5	3	2
135, nonfrozen.....	21	56.2	19.5	99	100	100	97
136, frozen.....	21	56.2	-----	2	3	3	1
137, nonfrozen.....	25	50.6	25.3	99	100	99	98
138, frozen.....	25	50.6	-----	57	72	62	35
139, nonfrozen.....	27	46.5	25.7	99	99	98	94
140, frozen.....	27	46.5	-----	42	60	80	22
141, nonfrozen.....	29	46.5	26.8	99	100	98	96
142, frozen.....	29	46.5	-----	17	66	63	9
143, nonfrozen.....	31	45.0	28.5	99	100	98	89
144, frozen.....	31	45.0	-----	10	84	89	0
145, nonfrozen ¹	33	43.5	29.9	98	99	98	98
146, frozen.....	33	43.5	-----	42	77	85	27
147, nonfrozen.....	35	38.7	30.7	99	100	100	93
148, frozen.....	35	38.7	-----	42	80	80	32
149, nonfrozen.....	38	34.1	30.2	100	100	99	95
150, frozen.....	38	34.1	-----	72	89	88	63
151, nonfrozen.....	41	-----	31.0	99	100	99	98
152, frozen.....	41	-----	-----	92	98	97	82

¹ Main part of field harvested.

The effect of freezing the kernels on their germination on November 24, 1923, is apparent in all of the stages of development studied. The germination of the nonfrozen samples was very high throughout the whole range. The germination tests carried out about a month later show a great increase in the germination of those frozen samples which had a moisture content of 50 per cent or less at the time of freezing. After aging for more than a year the germination of the frozen samples had decreased markedly, while the nonfrozen samples

decreased in germination only slightly or not at all. Table I shows very clearly the pronounced effect of aging on the germination of frozen wheat, the germination being at first relatively low, increasing to a maximum, and then decreasing, so that at the end of a year the germination is again relatively low. The germination of the wheat which was more mature at the time of frost was less affected by freezing than was the more immature wheat. It would seem from an examination of Table I that if frosted wheat is to be used for seed it should be tested for germination immediately before seeding. Comparisons of the effects produced by subjecting seeds to freezing temperatures should include aging experiments.

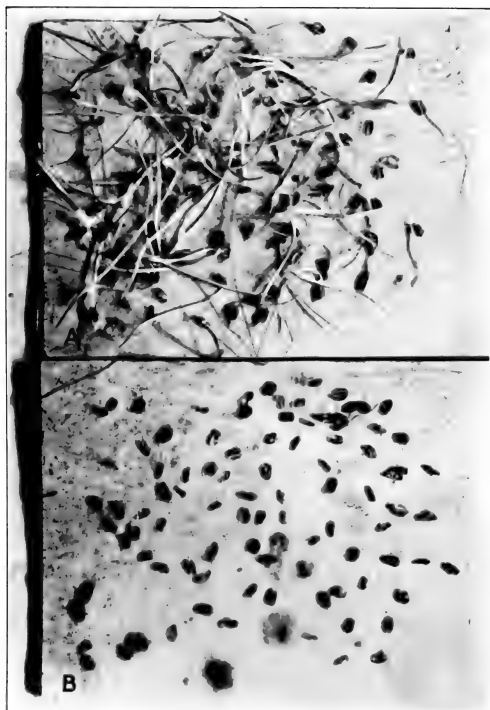
Kiesselbach and Ratcliff (9) studied the effect on germination of subjecting immature corn to freezing temperatures. Their results show clearly that at a given moisture content the injury increases as the freezing temperature is lowered, and that "death from freezing is directly related to the moisture content of the kernel and also to the duration of the exposure to cold."

The wheat used in this investigation was subjected to freezing temperatures considerably lower than wheat would normally encounter in the field. These low temperatures were chosen intentionally, for the reason that if no effect was produced under these conditions then no effect would be expected under less severe ones. The results indicate the desirability of investigating the effect of less severe freezing temperatures.

Attention is called to the almost complete germination of the nonfrozen wheat at all of the stages of development studied. Even when the kernel was only approximately 13 days old germination was practically complete. The investigations of Harlan and Pope (6) on the germination of barley harvested at different stages of growth are of interest in this connection. These investigators hand-pollinated Hannchen barley so that they knew the exact age of the kernel to within one hour. They found no germination on the fourth day, but on the fifth day 9 out of 10 kernels germinated. The dry matter content of the Hannchen barley at 6 days of age was given as about 5 mgms. Kiesselbach and Ratcliff (9), in studying seed corn production, found that "the power of germination is attained in about 20 days after fertilization."

The germination tests carried out by the ice-box method December 21, 1923, gave only slightly different results from the alternating-temperature method.

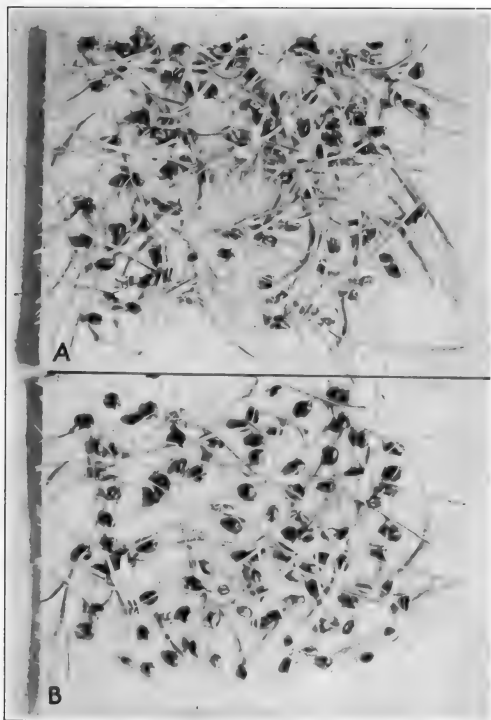
Photographs of the germinated kernels from the test of December 21, 1923, using the alternating-temperature method for samples 131 and 132, are given in Plate 2; for samples 137 and 138, in Plate 3; and for samples 149 and 150, in Plate 4. These plates give an indication of the probable strength of the plant produced. There is apparently a greater development in the more mature samples.



Germination tests of wheat made December 21, 1923, by the alternating-temperature method

- A. Sample No. 131, nonfrozen wheat germination, 95 per cent
- B. Sample No. 132, frozen wheat germination, 2 per cent

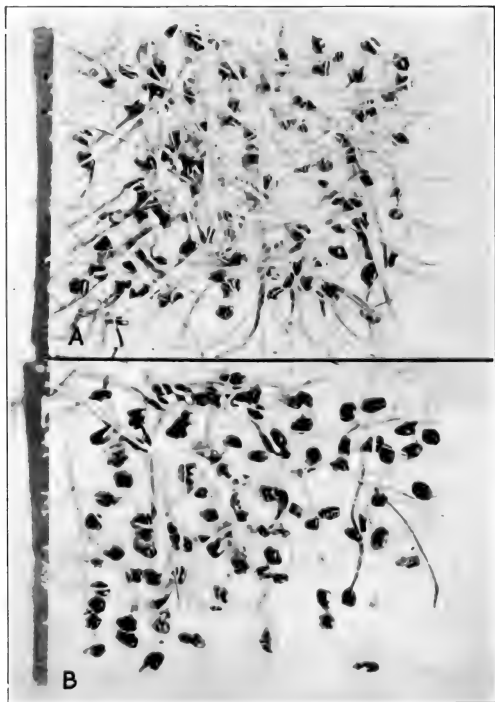
Approximate age of kernels, 13 days



Germination tests of wheat made December 21, 1923, by the alternating-temperature method

- A.— Sample No. 137, nonfrozen wheat germination, 100 per cent
B.— Sample No. 138, frozen wheat germination, 72 per cent

Approximate age of kernels, 25 days



Germination tests of wheat made December 21, 1923, by the alternating-temperature method

- A.—Sample No. 149, nonfrozen wheat germination, 100 per cent
- B.—Sample No. 150, frozen wheat germination, 80 per cent

Approximate age of kernels, 38 days

CONCLUSIONS

Very immature wheat shows almost perfect germination.

Freezing impairs the germination of wheat less the more mature the wheat is at the time of freezing.

The germination of frosted wheat is greatly affected by aging. The germination is at first relatively low, increases with time to a maximum, and then decreases to a low degree.

Frosted wheat should be tested for germination immediately before seeding.

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THE INFLUENCE OF NITRATE NITROGEN UPON THE PROTEIN CONTENT AND YIELD OF WHEAT¹

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REVIEW OF LITERATURE

The factors which influence the formation of protein in wheat have been studied by several investigators. From their results two theories appear worthy of consideration, (1) that the moisture content of the soil during the growing period is the controlling factor in protein formation, a high moisture content generally resulting in the production of wheat of low protein content, and (2) that the quantity of nitrate nitrogen present in the soil during the growth of the wheat is the most important factor in the production of a high protein content. No attempt will be made in this paper to give a complete review of the literature published.

INFLUENCE OF MOISTURE UPON PROTEIN FORMATION

Most of the results of the work of the early investigators indicate that moisture is the controlling factor in protein formation, an opinion shared by most farmers in the irrigated sections of Montana. The general opinion seems to be that when the moisture is increased a wheat of low protein content is produced. Thatcher (6)² found that certain factors of climate, such as rainfall and temperature, have a decided influence on protein formation, and he concluded that the protein content of wheat in eastern Washington decreases with an increase of rainfall. He also calls attention to the length of the growing period as a factor in protein production. Widtsoe and Stewart (7) found that a variation in the quantity of irrigation water caused a difference in the protein content of wheat. From their analytical data they concluded that the protein content is lowered with an increase of irrigation water. Harris (2), working in the greenhouse where controlled conditions were possible, found that the percentage of nitrogen in both grain and straw was influenced by the quantity of moisture in the soil. The highest protein content was obtained from wheat grown in soil of low moisture content, and the lower protein content from soil containing the higher percentages of moisture. Harris found further that fertilizers high in nitrogen brought about an increase in the nitrogen content of wheat crops.

INFLUENCE OF NITRATE NITROGEN ON PROTEIN FORMATION

Headden (3) conducted an extensive investigation of the factors which influence protein formation, and he was among the first to make a study of the available nitrogen present in the soil during plant growth. In addition, he studied the influence of a varying

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² Reference is made by number (italic) to "Literature cited," p. 1199.

quantity of irrigation water applied to the land during the growing season. In speaking of the wheat grown in Colorado on land irrigated with 1 and with 2 feet of water, he states that no results were secured which showed conclusively that the different amounts of water applied made any difference in the weight of the wheat per bushel or in the composition of the grain. Jones, Colver, and Fishburn (4) also made a study of the factors influencing wheat protein formation. In their conclusions they state that growers and millers are wrong in assuming that low protein wheats necessarily result from the practice of irrigation and that, as a matter of fact, irrigation is not the controlling factor in determining the protein content of the harvested grain. They state further that a better quality of grain is possible when wheat is brought into rotation with alfalfa or red clover, since the activity of nitrifying organisms in the sod of these legumes when turned under provide a substantially larger supply of available nitrogen for growing wheat plants.

Neidig and Snyder (5) made an extensive investigation of the factors which influence the formation of protein. Their studies were confined to pot experiments with different types of soil and the application of different fertilizers. They found that the available nitrogen in the soil directly affected both the quantity of protein in the grain and the yield of wheat under the conditions of their experiments. They state that climate also plays an important part in the quality and yield of wheat.

In some of the publications on the influence of moisture upon protein formation there seems to be a lack of data relative to the quantity of nitrates in the soil during the period over which the experiments extended.

EXPERIMENTS IN MONTANA

From 1911 to 1917, inclusive, an investigation was conducted at the Montana Experiment Station for the purpose of studying the factors which influence nitrate formation in the soil. As will be shown, the results obtained have a direct bearing on the subject of protein formation. The experiments were made in a dry-land section of Gallatin Valley having an average rainfall of nearly 20 inches. The soil is a silt loam, classified as Yakima silt loam. It is of alluvial origin, at one time the bottom of an old lake bed, and is especially rich in all of the plant food elements. The total nitrogen averaged about 0.25 per cent. This soil was cultivated first in 1910, when it was laid out in twentieth-acre plots. The data used in this article are from plots cropped continuously with both winter and spring wheat and from plots growing winter and spring wheat under alternate fallow and crop. The plots growing continuous crops of wheat were plowed either in the fall or early spring. The plots summer-fallowed were plowed in the early spring and cultivated throughout the growing season for the purpose of conserving moisture and keeping the land free from weeds.

SAMPLING THE SOIL

Soil samples were taken about every two weeks for the purpose of determining the moisture content and the quantity of nitrates and nitrites present. These samples represented the first, second, third,

fourth, and fifth feet of soil, except the first sampling in the spring, the midsummer sampling, and the last sampling in the fall when samples were taken to a depth of 10 feet. The crops were harvested in the autumn and a careful record was made at the time of threshing of the pounds per plot of both grain and straw. Samples of both were taken from all plots and analyzed for total nitrogen.

TABLE I.—Yield in pounds of grain and straw, percentage of nitrogen in the crop, pounds of nitrogen removed by the crop, average percentage of moisture found in the first 3 feet of soil during the growing season, the average parts per million of nitrate nitrogen, and the concentration of the nitrate nitrogen in the soil solution

PART 1. WINTER WHEAT—CONTINUOUS CROPPING

Year	Plot	Yields, in pounds		Percentage of nitrogen in—		Pounds of nitrogen removed by—		Total nitrogen removed	Percentage of water in first 3 feet of soil	P. P M. nitrate nitrogen in first 3 feet of soil	Concentration of nitrate nitrogen in soil solution	Dates of sampling	
		Grain	Straw	Grain	Straw	Grain	Straw					First	Last
1911--	1	95.0	259	2.30	-----	2.18	-----	-----	15.55	2.05	11.3	May 2	July 11
1912--	1	102.6	149	2.30	0.44	2.36	0.66	3.02	16.34	6.38	33.6	Apr. 29	July 26
1913--	1	65.2	118	1.78	.40	1.16	.47	1.63	12.76	2.06	14.2	May 6	July 28
1914--	1	71.3	57.9	2.08	.59	1.48	.34	1.82	15.22	2.23	12.7	May 9	July 21
1915--	1	82.7	131.3	2.13	.34	1.76	.45	2.21	20.58	2.26	9.1	May 10	July 20
1916--	1	72.2	94	1.92	.43	1.38	.40	1.78	20.05	2.03	8.4	Apr. 26	Aug. 7
1917--	1	21.8	35	2.10	.67	.46	.23	.69	18.49	2.14	9.8	May 8	July 17
Average--		72.9	120.7	2.09	.48	1.54	0.42	1.86	17.00	2.73	14.2	-----	-----

SPRING WHEAT—CONTINUOUS CROPPING

1911--	2	61.8	128.0	1.50	0.69	0.93	0.88	1.84	16.34	2.51	13.2	May 16	Aug. 15
1912--	2	101.7	150.0	1.91	.34	1.94	.51	2.47	15.91	4.29	23.2	May 21	Sept. 4
1913--	2	66.2	123.7	1.98	.42	1.31	.52	1.83	13.91	1.95	12.3	May 23	Aug. 7
1914--	2	38.4	104.5	2.66	.63	1.02	.66	1.68	16.25	2.04	10.8	June 3	Aug. 17
1915--	2	50.0	117.6	2.12	.58	1.06	.68	1.75	19.25	1.85	8.0	May 24	Aug. 24
1916--	2	77.0	87.0	2.02	.28	1.55	.24	1.85	20.02	2.25	9.4	May 23	Aug. 7
1917--	2	30.0	57.0	2.09	.57	.62	.32	.95	16.39	1.48	7.7	May 24	Aug. 9
Average--		60.7	109.7	2.04	.50	1.20	.54	1.78	16.87	2.34	12.1	-----	-----

PART 2. WINTER WHEAT—ALTERNATE FALLOW AND CROP

1911--	12	103.5	284.0	2.30	0.51	2.38	1.45	3.83	15.35	4.06	22.9	May 6	July 15
1912--	16	158.6	316.3	2.36	.53	3.74	1.68	5.42	16.34	12.68	66.6	May 9	July 29
1913--	12	149.8	206.4	2.40	.44	3.60	.91	4.51	15.26	5.69	32.4	May 9	July 28
1914--	16	105.0	326.8	2.70	.57	2.84	1.86	4.72	15.76	2.13	11.7	May 6	July 22
1915--	12	156.3	342.0	2.06	.32	3.22	1.09	4.31	19.34	2.43	10.5	May 13	July 12
1916--	16	117.0	161.5	2.63	.51	3.08	.82	3.90	22.49	5.17	18.8	May 2	Aug. 7
1917--	12	53.2	118.8	2.90	.76	1.54	.90	2.45	17.98	4.33	20.4	May 8	July 18
Average--		120.5	250.8	2.48	.52	2.90	1.24	4.16	17.50	5.21	26.2	-----	-----

SPRING WHEAT—ALTERNATE FALLOW AND CROP

1911--	17	99.8	190.0	2.30	0.51	2.29	0.97	3.26	17.03	5.75	28.8	May 16	Aug. 17
1912--	13	145.3	210.9	2.94	.78	4.27	1.65	5.92	14.43	7.79	47.3	May 23	Sept. 4
1913--	17	129.7	259.3	2.75	.41	3.57	1.06	4.62	13.91	5.57	35.1	May 23	Aug. 7
1914--	13	61.4	186.0	3.28	.89	2.01	1.65	3.66	17.59	4.08	19.5	May 21	Aug. 3
1915--	17	91.8	284.4	2.64	.64	2.42	1.82	4.25	19.25	3.84	16.7	May 26	Aug. 24
1916--	13	117.8	172.0	2.85	.46	3.35	.79	4.14	21.01	7.79	30.6	May 23	Aug. 5
1917--	17	40.8	73.0	2.84	.69	1.16	.51	1.67	16.39	7.03	37.0	May 24	Aug. 15
Average--		98.1	196.5	2.80	.63	2.72	1.21	3.93	17.09	5.98	30.7	-----	-----

Table I gives the yield in pounds of the wheat and straw. It also shows the percentage of nitrogen in the crop and the total number of pounds of nitrogen removed by the crop. The average percentage of moisture found in the first 3 feet of soil during the growing season, the average parts per million of nitrate nitrogen, and the concentration of the nitrate nitrogen in the soil solution are also included. The moisture and nitrate data are based on a 3-foot average on the assumption that that found in the soil at greater depths has but little influence upon yield or quality of wheat. An inspection of part 1 of this table shows a variation in yields which can hardly be attributed to a difference in the moisture content of the soil, or can it be attributed entirely to a variation in the different amounts of available nitrogen present. Table II, giving monthly precipitation and mean monthly temperatures, shows a variation in both of these factors which must be considered. While both moisture and nitrates affect quality and yield, there are other factors, such as temperature, sunshine, and length of growing season, which must also be considered. Although a comparison can not be made of the yield and protein content of the grain and straw in relation to the moisture found in the soil for the different years on the continuously cropped and alternate fallow and cropped plots, yet it is but fair to assume that comparisons can be made for the same year when climatic conditions are necessarily the same.

TABLE II.—*Monthly precipitation and monthly mean temperature records for Bozeman, Mont., from April, 1911, to August, 1917*

Year	Monthly precipitation						Monthly mean temperature				
	April	May	June	July	Aug- ust	Total	April	May	June	July	Au- gust
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>
1911.....	1.57	3.56	3.06	0.84	1.52	10.55	38.5	47.4	58.7	60.8	58.3
1912.....	3.59	2.66	2.69	1.91	1.63	12.48	31.3	49.2	58.7	59.9	58.4
1913.....	1.65	2.54	3.19	2.17	1.80	11.33	42.6	49.0	58.1	60.1	64.3
1914.....	2.02	2.05	3.54	1.28	.11	9.00	40.6	50.6	55.4	65.1	61.8
1915.....	2.60	3.67	4.44	3.91	.38	15.00	48.6	46.8	51.4	57.3	64.2
1916.....	2.10	2.99	2.33	1.58	1.38	10.38	40.8	43.0	53.1	64.2	60.9
1917.....	1.72	2.37	2.46	.36	.53	7.44	35.4	46.9	55.1	68.8	63.4
Average.....	2.17	2.83	3.10	1.72	1.05	10.88	41.1	47.5	55.8	62.3	61.6
Normal ^a	1.93	2.82	2.88	1.32	.93	9.88	41.3	49.4	57.2	63.7	62.9

^a 35-year average.

RESULTS FROM WINTER WHEAT

By referring to Table I it will be seen that the average yield of winter wheat grown on the continuously cropped plots was 72.9 pounds; the average yield of winter wheat on the alternate fallow and crop plots was 120.5 pounds. The average moisture content on the continuously cropped plots, to a depth of 3 feet, was 17.00 per cent; the moisture content on the alternate fallow and crop was 17.57 per cent. The average nitrogen content of the grain grown on the continuously cropped plots for the seven years was 2.09 per cent; that grown on the alternate fallow and crop plots was 2.48 per cent, a decided increase over the continuously cropped plots which had a lower percentage of moisture in the soil.

RESULTS FROM SPRING WHEAT

The plot growing spring wheat, continuously cropped, also shows a lower moisture content of the soil to a depth of 3 feet and a lower protein content of the grain. Here again the soil containing the higher percentage of moisture produced the largest yields and a grain of higher protein content. In studying Table I it must be observed that in each year the plots containing the highest percentages of available nitrogen produced wheat of a higher protein content, one exception only being noted. This occurred in 1915 when the alternate fallow and crop plot produced grain having a nitrogen content of 2.06 per cent and the continuously cropped plot produced grain having a nitrogen content of 2.13 per cent. The results of that year's experiment thus show but very little difference in the available nitrogen, though the difference is slightly in favor of the alternate fallow and crop. An examination of the writer's data indicates that in that year the average available nitrogen in the first foot of soil was 2.24 parts per million for the continuous cropping and 2.19 for the alternate fallow and crop plot. It is possible that the available nitrogen in the first foot exerted the greatest influence on protein formation, but the difference in the quantity of nitrates in the soil was too slight to account for the difference in amount of protein formed in the wheat. The fact that the alternate fallow and crop plot gave not only a higher average yield but also a higher average percentage of nitrogen content in both grain and straw shows at least the influence which available nitrogen exerts on the crop and its composition.

MOISTURE AND NITRATES IN SOIL AS RELATED TO YIELDS AND PROTEIN CONTENT

WINTER WHEAT

In order to present more clearly the influence of moisture and nitrate nitrogen in the soil upon yield and protein formation of wheat, the data have been charted showing the difference in the moisture content of the soil at different periods of plant growth and also the different amounts of nitrate nitrogen for the same periods (figs. 1 and 2). Figure 1 shows a variation of less than 1.5 per cent in the moisture content of the soil from the time the winter wheat began to grow in the spring until it was ready to harvest. The greatest variation occurred at the time of the second sampling, May 23, and before the winter wheat had made sufficient growth for taking moisture from the soil at its maximum rate. After the sampling on this date, there is no period in the growth of the wheat when the moisture content varies enough to account for any difference in the yield or composition of the grain. These differences must then be explained by some other cause. Figure 2 indicates that there is a wide variation in parts per million of nitrate nitrogen in the soil for the same plots continuously and alternately cropped. The continuously cropped plots show a lower percentage of nitrate nitrogen at each sampling than do the alternate fallow and crop. The very fact that both grain and straw contain a higher percentage of nitrogen suggests very strongly that the difference in the quantity of nitrate nitrogen present in the soil is also largely responsible for the difference in yields.

SPRING WHEAT

For spring wheat Figure 1 shows but little difference in the moisture content of the plots continuously cropped and those growing spring wheat alternating with fallow. The difference up to June 28 is not sufficient to cause any difference in the rate of growth. It must be noted, however, that after June 28 there was a more decided drop in the moisture content of the alternate fallow and crop plots than in the plots continuously cropped. It is quite possible that the greater

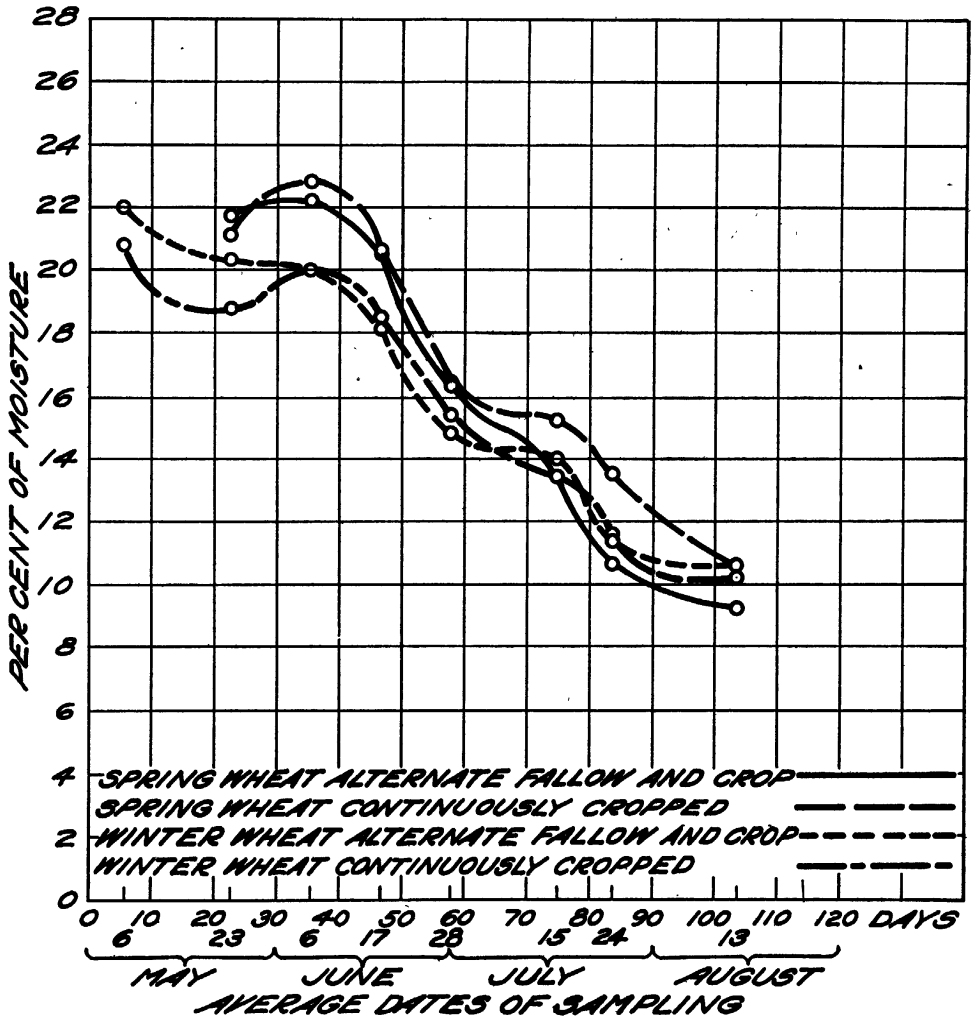


FIG. 1.—Moisture content of the soil in several plots at different periods of plant growth

growth of the spring wheat, as shown by the yields, brought about this change. If this be true, then it is quite certain that some factor in addition to moisture was responsible for the difference in plant growth. This difference can not be attributed to any difference in climatic conditions. A study of Figure 2 shows that the summer-fallowed plot contained much more nitrate nitrogen than the plot continuously cropped. In Table III the plot yields are calculated to bushels per acre, and these calculations show that the average of seven years' yield of winter wheat is $24\frac{1}{2}$ bushels on the continuously cropped land and $40\frac{1}{6}$ bushels on the alternate fallow and crop land.

The seven-year average on continuously cropped land shows a yield of 20¼ bushels of spring wheat as against 32½ bushels on the alternate fallow and crop land. The average protein content of both spring and winter wheat produced on the continuously cropped land is much lower than it is in the spring and winter wheat produced on the alternate fallow and crop.

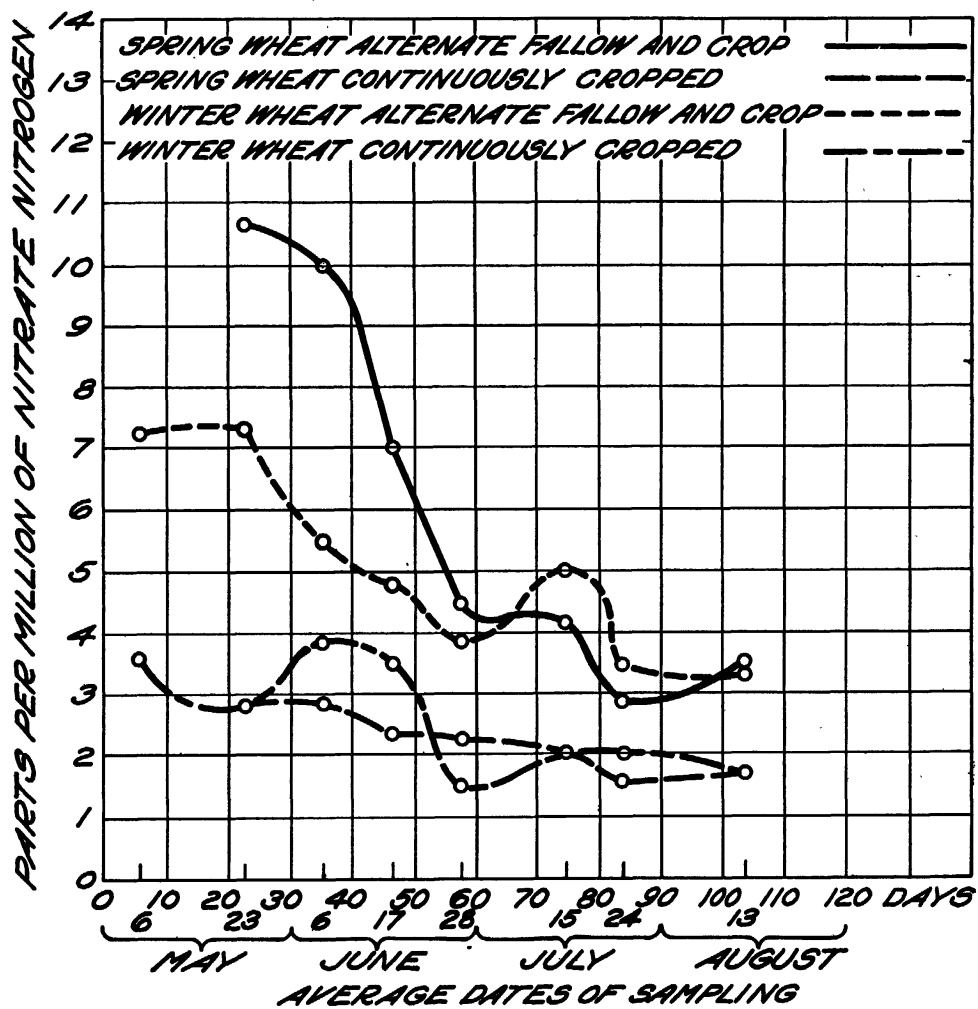


FIG. 2.—Nitrate nitrogen found in the soil of several plots at different periods of plant growth

TABLE III.—Yield per acre and protein content of wheat on plots continuously cropped and plots alternately fallow and cropped (seven-year average)

	Plots continuously cropped		Plots alternately fallow and cropped	
	Bushels	Percentage of protein	Bushels	Percentage of protein
Winter wheat.....	24½	11. 91	40½	14. 13
Spring wheat.....	20¼	11. 63	32½	15. 90

From a study of the graphs showing moisture and nitrates it seems hardly possible that any of the differences which appear in the

moisture content of the soil to a depth of 3 feet could be the controlling factor in bringing about the difference both in yield and in protein formation. The fact that there is more available nitrogen in the summer-fallowed plots than in those continuously cropped indicates quite strongly that the available nitrogen in the soil is the more important factor.

EFFECT OF CLIMATIC CONDITIONS

The yearly variations in yields and percentages of nitrogen found in grain and straw are undoubtedly influenced by climatic conditions. An inspection of Table II shows that the year 1917 was the driest year recorded during the seven-year investigation, and it shows also that the temperatures for July and August of that year were considerably above the average. A lack of moisture accompanied by high temperatures during these months was undoubtedly the controlling factor

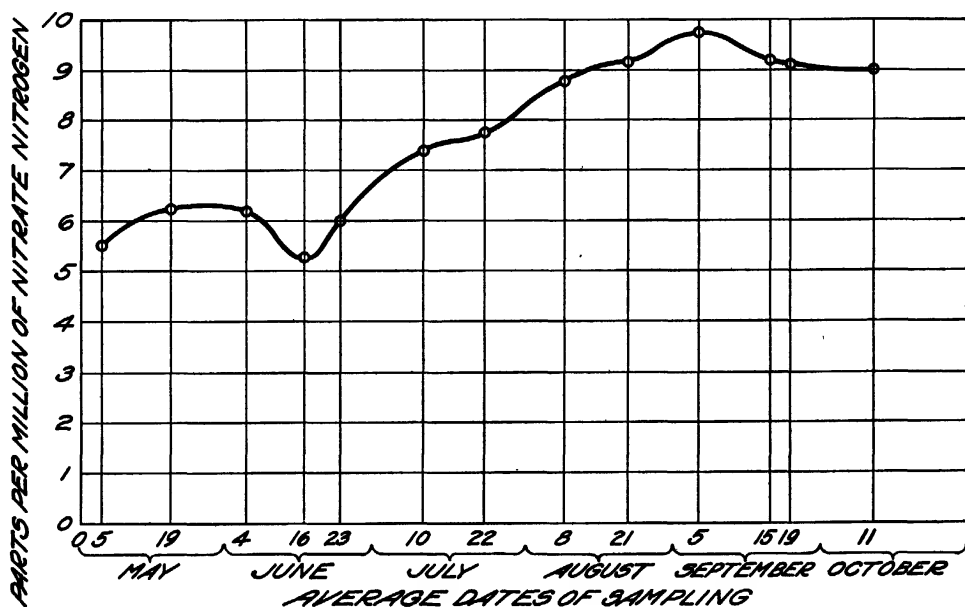


FIG. 3.—Nitrate nitrogen found in the soil of four summer-fallow plots at different periods of plant growth

in producing small yields. The yields for 1914 were quite certainly affected by a low precipitation during July and August and a high temperature during July. The year 1915 gave, in general, very good yields. The precipitation for the months of June and July was considerably above the average, but the temperature for the same months was below normal. These conditions were favorable for plant growth and were important factors in producing larger yields that year. There does not seem, however, to be any constant ratio between the protein content of the wheat and the yield on the continuously cropped plots for the different years, nor is there any decided difference in the protein content of the wheat grown on the alternate fallow and crop plots in favor of the dry years. The most pronounced difference was with spring wheat in 1914.

The data on the formation of nitrate nitrogen in the soil, given in Figure 3, show that there was a decided loss in June in the first 3 feet of soil. It is possible that the ground water carried some of the

nitrates below this depth. Table IV shows the average loss of nitrate nitrogen for two plots at the time when the greatest loss shown in Figure 3 occurred. This, however, represents a loss of nitrate nitrogen to a depth of 5 feet. Unpublished data of the writer show that there was no leaching of nitrates below the fifth foot for the years 1911, 1912, 1913, and 1917. A slight leaching was noted in the years 1914, 1915, and 1916. This leaching caused an increase in moisture of from 1 to 2 per cent in the fifth foot. It is hardly possible, however, that this slight degree of leaching brought about the reduction apparent in Table IV. The table shows a gain in nitrate nitrogen between the first and second sampling for the year 1916. It must be noted, however, that on May 23, 1.35 parts per million of nitrate nitrogen was found in the soil, the smallest amount shown in the table.

TABLE IV.—Loss or gain in parts per million of nitrate nitrogen in the soil to a depth of 5 feet.

Year	Plot No.	Date of first sampling	Parts per million of nitrate nitrogen	Date of second sampling	Parts per million of nitrate nitrogen	Percentage loss	Loss in pounds per acre to a depth of 5 feet
1911-----	13 and 16	June 2	5.00	June 13	2.68	46.1	40.6
1912-----	12	June 3	6.58	June 12	5.33	19.0	21.9
1913-----	13 and 16	do-----	11.61	July 1	9.07	18.2	44.4
1914-----	12 and 17	May 21	7.68	June 11	4.86	31.2	49.3
1915-----	13 and 16	May 26	11.55	June 9	6.62	43.8	86.3
1916-----	12 and 17	May 23	1.35	do-----	2.61	+37.6	* 22.1
1917-----	13 and 16	May 24	6.61	June 13	5.49	14.1	19.6

* Gain.

NOTE.—The percentages gain or loss of nitrate nitrogen given in this table were obtained by taking an average of the percentages of two plots; 17,500,000 pounds was the weight of the soil used in calculating the loss per acre.

Meteorological data, not included in this article, show that 0.97 inch of precipitation fell as snow between May 9 and May 13. Between May 19 and May 22 there was 1 inch of rain. This cold, wet spell was responsible for the reduction of nitrates prior to the sampling on May 23, a fact which is clearly evident from unpublished data showing that on May 1 there had been 3.97 parts per million of nitrate nitrogen. This is one year when the reduction took place before the first date of sampling, shown in Table IV.

If such conditions occur in soil receiving a monthly precipitation of something like 3 to 4 inches, it is reasonable to suppose that irrigation would have a decidedly greater influence in bringing about a loss of nitrogen either by carrying the nitrates into some porous substratum where they would be lost or, if there is no such substratum, to depths where denitrification would occur. It is altogether probable that irrigation favors even a greater reduction in the amount of available nitrogen than takes place under normal rainfall conditions. Hall (1) states that—

a comparison of the nitric nitrogen in the first 5 feet of a nonirrigated cultivated soil with that in an irrigated cultivated soil shows the nonirrigated soil of the same type to be much superior in nitrate content, although the irrigated soil does not get sufficient water to carry the nitrates to the fourth foot.

If irrigation such as that maintained by Hall caused a decrease in nitrate nitrogen formation, it is quite certain that excessive irrigation would cause a greater decrease. Spring rains also reduce the quantity of nitrate nitrogen in the soil. It is quite possible that an increase in moisture, either by natural causes or by irrigation, decreases the protein content of wheat indirectly through its reduction of the nitrates in the soil.

The difference in the yields obtained from continually cropped and summer-fallowed plots corresponds very closely to that obtained by farmers on the dry land in the same locality. Even in the irrigated sections of Gallatin Valley the farmers find a wide difference in the yields of grain when grown continuously or when grown on summer-fallowed land. Nor can the difference in the grain yields obtained by farmers on irrigated land by these two methods of cropping be attributed to a difference in moisture conditions of the soil. The farmers of the irrigated districts abandoned the general custom of alternate fallow and crop several years ago and adopted a system of rotation with clover or some other legume as one of the crops. This practice has kept up the organic content of the soil and also the yields of grain, in marked contrast to the results obtained in former years when some farmers attempted to grow grain continuously.

CONCLUSIONS

Plots alternated with fallow and crop produce more spring and winter wheat per acre than plots of the same soil type cropped continuously.

Plots alternated with fallow and crop contained on an average more moisture to a depth of 3 feet than the plots cropped continuously. The greatest difference in moisture content occurred with spring wheat when the plants were making their most rapid growth. With winter wheat the greatest difference in moisture content occurred at a time when the soil was well supplied with moisture and before the plants were making their most rapid growth.

Plots alternated with fallow and crop contain more nitrate nitrogen during the cropped year than those cropped continuously. This extra supply of available nitrogen undoubtedly accounts for the alternate fallow and cropped plots producing straw and grain of a higher nitrogen content than those cropped continuously.

Nitrate nitrogen in the alternate fallow and crop plots shows a greater decrease in parts per million in the cropped years than does the continuously cropped plots. This decrease started before the wheat plants were large enough to take up much plant food from the soil and must be explained largely on the theory of leaching. A continuation of the decrease may be explained on the supposition that the wheat plants were taking nitrates from the soil faster than they were formed.

The higher yields and protein content of wheat grown on the alternate fallow and crop plots over those on plots cropped continuously can not be explained from the standpoint of moisture. The fact that the fallow plots always produced larger yields and grain of a higher protein content than the plots cropped continuously indicates that nitrate nitrogen is certainly a factor, and probably the greatest factor, in controlling yields and quality of wheat in Montana.

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